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<b>(21) International Application Number:</b> PCT/US99/11702 <b>(22) International Filing Date:</b> 27 May 1999 (27.05.99)  <b>(30) Priority Data:</b> 60/087,577      1 June 1998 (01.06.98)      US  <b>(71) Applicant (for all designated States except US):</b> OR-THO-McNEIL PHARMACEUTICAL, INC. [US/US]; U.S. Route 202, Raritan, NJ 08869 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> REITZ, Allen, B. [US/US]; 109 Grenbrier Road, Lansdale, PA 19446 (US). DEMETER, David, A. [US/US]; 10657 Blackthorn Court, Fishers, IN 46038 (US). LEE, Daniel, H., S. [CA/US]; 38 Trailwood Drive, Northhampton, PA 18966 (US). WANG, Hoau-Yan [US/US]; 149 Kalos Street, Philadelphia, PA 19128 (US). CHEN, Robert, H. [US/US]; 39 Beverly Drive, Belle Mead, NJ 08502 (US). ROSS, Tina, Morgan [US/US]; 11 Culp Road, Audubon, PA 19403 (US). SCOTT, Malcolm, K. [US/US]; 1036 Beverly Way, Lansdale, PA 19446 (US). PLATA-SALAMAN, Carlos, R. [US/US]; 1313 Squire Drive, Ambler, PA 19002 (US).		<b>(74) Agents:</b> CIAMPORCERO, Audley, A., Jr. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08903-7003 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> METHOD FOR TREATING NEURODEGENERATIVE DISORDERS  <b>(57) Abstract</b> <p>The invention is directed to a method of treating a neurodegenerative disorder in a subject in need thereof which comprises administering to the subject an amount of a compound effective to inhibit the interaction of amyloid-beta with alpha-7 nicotinic acetylcholine receptors.</p>		

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Title of the Invention

Method for Treating Neurodegenerative Disorders

5    Cross-Reference to Related Application

This application claims priority from United States provisional application Serial No. 60/087,577, filed June 1, 1998, the contents of which are hereby incorporated by reference.

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Field of the Invention

The present invention provides a method for treating neurodegenerative disorders. More particularly, a method for treating neurodegenerative disorders (e.g., Alzheimer's disease) by inhibiting the interaction of amyloid beta with alpha-7 nicotinic acetylcholine receptors.

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Background of the Invention

20       Neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) afflict humanity with great suffering and financial loss. AD is characterized by neurofibrillary tangles, neuritic plaques, and neuronal cell death. AD appears as either the familial, early onset (<60 yrs) or late-onset (>60 yrs) forms, with the latter being more prevalent. AD is the major cause of age-related dementia and cognitive impairment (Wisniewski, T.; Ghiso, J.; Frangione, B. *Neurobiol. of Disease* **1997**, 4, 313-328). The amyloid precursor protein (APP),  $\beta$ -amyloid<sub>1-40</sub> ( $A\beta_{1-40}$ ), and  $\beta$ -amyloid<sub>1-42</sub> ( $A\beta_{1-42}$ ) are keenly involved in the pathology of AD. The  $A\beta$  peptides are derived from APP by proteolytic processing. Dramatic evidence implicating the  $A\beta$  peptides, particularly  $A\beta_{1-42}$ , in AD comes from various recently identified mutations accounting for certain types of inherited AD. Such mutations in the presenilin (PS1 and PS2) genes are

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probably the cause of the most frequent form of familial, early-onset AD (Rogaev, E. I. *Molecular Biology* **1998**, 32, 58). In these cases, as with APP mutations, more A $\beta$ <sub>1-42</sub> is observed relative to A $\beta$ <sub>1-40</sub>. Extensive studies have shown that A $\beta$ <sub>1-42</sub> has a greater ability than A $\beta$ <sub>1-40</sub> to aggregate into the amyloid fibrils that constitute the plaques characteristic of AD (Lansbury, P. T., Jr. *Accts. Chem. Res.* **1996**, 29, 317). Even though A $\beta$ <sub>1-40</sub> is generally present to a much larger degree in the cerebrospinal fluid than A $\beta$ <sub>1-42</sub>, it is A $\beta$ <sub>1-42</sub> which is the major A $\beta$  peptide found in AD plaques.

10        The A $\beta$  peptides can inhibit cholinergic neurotransmitter function independent of neurotoxicity (Auld, D. S.; Kar, S.; Quirion, R. *Trends Neurosci.* **1998**, 21, 43). A $\beta$  peptides bind to a number of natural substances such as apoE3, apoE4, apoJ, transthyretin, and albumin. In addition, A $\beta$  has been reported to interact with a membrane-bound receptor  
15        for advanced glycation end products and to the class A scavenger receptor (SR) associated with the production of reactive oxygen species. Stimulation of the alpha-7 subtype of the nicotinic acetylcholine receptors (nAChRs) can protect neurons against A $\beta$  cytotoxicity (Kihara, T. et al. *Ann. Neurol.* **1997**, 42, 159). Also, a set of compounds that activate nAChRs,  
20        especially of the alpha-7 subtype, have been found to have *in vivo* activity in models of cognition enhancement (US Patent No. 5,741,802, issued April 21, 1998).

25        We now describe specific binding of A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> to the alpha-7 subtype of nAChRs. This new finding has broad ramifications for the etiology and treatment of AD. nAChRs are members of the ligand-gated ion channel family and appear to be formed from five protein subunits associating together around a central pore (Lindstrom, J. *Molecular Neurobiology* **1997**, 15, 193). These subunits include  $\alpha$ 1- $\alpha$ 9,  $\beta$ 1- $\beta$ 4,  $\gamma$ ,  $\delta$ ,  
30        and  $\epsilon$ . The  $\alpha$ 7 subtype forms functional homomers which bind to  $\alpha$ -bungarotoxin, a 75-amino acid peptide, with high affinity (0.65-1.7 nM K<sub>d</sub>)

and nicotine with relatively low affinity (ca. micromolar  $K_d$ ) (Holladay, M. W.; Dart, M. J.; Lynch, J. K. *J. Med. Chem.* **1997**, 40, 4169).

Compounds which block the aggregation of A $\beta$  peptides are potentially  
5 useful drugs for the treatment of AD. For example, rifampicin inhibits A $\beta$   
aggregation and neurotoxicity and may show an effect *in vivo* in diminishing  
plaque burden when compared with age-matched controls (Tomiya, T. et  
al. *J. Biol. Chem.* **1996**, 271, 6839). In order to block the interaction of the  
A $\beta$  peptides with  $\alpha 7$  nAChRs, compounds can be found to either bind to  $\alpha 7$   
10 nAChRs, to A $\beta$  itself, or to both. Any of these mechanisms of action would  
be expected to provide significant protection against A $\beta$ -mediated  
neurotoxicity and inhibition of cholinergic functioning mediated by nAChRs  
and be extremely useful for the treatment of AD. The binding of A $\beta_{1-42}$  to  
alpha-7 nAChRs provides a seed for crystallization or deposition of A $\beta$  into  
15 insoluble deposits, which have the potential to grow into the fibrillar amyloid  
deposits characteristic of AD. Therefore, blocking the interaction of A $\beta_{1-42}$   
with alpha-7 nAChRs should reduce the amount of insoluble aggregated A $\beta$   
that is formed, and thus prevent the neurotoxicity and pathology associated  
with such aggregated amyloid deposits.

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Accordingly, it is an object of the invention to provide a method for treating  
neurodegenerative disorders by inhibiting the binding of amyloid beta peptides to  
alpha-7 nicotinic acetylcholine receptors. It is a further object of the invention to  
provide a method for treating Alzheimer's disease and/or for slowing the  
25 progression of Alzheimer's disease by inhibiting the binding of amyloid beta  
peptides to alpha-7 nicotinic acetylcholine receptors. Another object of the  
invention is to provide a predictive method, a method for diagnosis, a method  
to monitor prognosis, a method to monitor the progression, and a method to  
monitor the therapeutic efficacy for any therapeutic intervention used in  
30 Alzheimer's disease. Still another object of the invention is to provide a method  
for identifying compounds which inhibit the binding of A $\beta$  peptides with  $\alpha 7$   
nAChRs, either by binding to A $\beta$  peptides or to  $\alpha 7$  nAChRs.

Summary of the Invention

The present invention is directed to a method of treating a  
5 neurodegenerative disorder in a subject (preferably, a human) in need thereof  
which comprises administering to the subject an amount of a compound  
effective to inhibit the binding of an amyloid beta peptide, preferably  $A\beta_{1-42}$ , to  
alpha-7 nAChRs, preferably, human alpha-7 nAChRs. Since alpha-8 and  
alpha-9 nAChRs are similar with respect to structure and function to alpha-7  
10 nAChRs, it is possible that blocking the interaction of  $\beta$ -amyloid with alpha-8  
and alpha-9 nAChRs would have therapeutic benefit as well.

Neurodegenerative disorders included within the methods of the present  
invention include, but are not limited to, Alzheimer's disease, Pick's disease,  
15 diffuse Lewy body disease, progressive supranuclear palsy (Steel-Richardson  
syndrome), multisystem degeneration (Shy-Drager syndrome), motor neuron  
diseases including amyotrophic lateral sclerosis, degenerative ataxias, cortical  
basal degeneration, ALS–Parkinson's–Dementia complex of Guam, subacute  
sclerosing panencephalitis, Huntington's disease, Parkinson's disease,  
20 synucleinopathies, primary progressive aphasia, striatonigral degeneration,  
Machado-Joseph disease/spinocerebellar ataxia type 3 and  
olivopontocerebellar degenerations, Gilles De La Tourette's disease, bulbar  
and pseudobulbar palsy, spinal and spinobulbar muscular atrophy (Kennedy's  
disease), primary lateral sclerosis, familial spastic paraplegia, Werdnig-  
25 Hoffmann disease, Kugelberg-Welander disease, Tay-Sach's disease,  
Sandhoff disease, familial spastic disease, Wohlfart-Kugelberg-Welander  
disease, spastic paraparesis, progressive multifocal leukoencephalopathy, and  
prion diseases (including Creutzfeldt-Jakob, Gerstmann-Sträussler-Scheinker  
disease, Kuru and fatal familial insomnia).

30 Other conditions also included within the methods of the present invention  
include age-related dementia and other dementias and conditions with memory

loss including vascular dementia, diffuse white matter disease (Binswanger's disease), dementia of endocrine or metabolic origin, dementia of head trauma and diffuse brain damage, dementia pugilistica and frontal lobe dementia. Also other neurodegenerative disorders resulting from cerebral ischemia or infarction

5 including embolic occlusion and thrombotic occlusion as well as intracranial hemorrhage of any type (including, but not limited to, epidural, subdural, subarachnoid and intracerebral), and intracranial and intravertebral lesions (including, but not limited to, contusion, penetration, shear, compression and laceration).

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Preferably, the neurodegenerative disorder is selected from Alzheimer's disease, Parkinson's disease, Tourette's syndrome, amyotrophic lateral sclerosis, age-related memory loss, senility and age-related dementia, most preferably, the neurodegenerative disorder is Alzheimer's disease. Because, most preferably, the

15 neurodegenerative disorder is Alzheimer's disease, also defined as an amyloidosis, other conditions within the methods of the present invention include other amyloidosis which share features including, but not limited to, hereditary cerebral angiopathy, nonneuropathic hereditary amyloid, Down's syndrome, macroglobulinemia, secondary familial Mediterranean fever, Muckle-Wells

20 syndrome, multiple myeloma, pancreatic- and cardiac-related amyloidosis, chronic hemodialysis arthropathy, and Finnish and Iowa amyloidosis.

In one embodiment of the invention is a method of treating and/or preventing dementia in an Alzheimer's patient (as well as a method for treating

25 and/or preventing other clinical manifestations of Alzheimer's disease that include, but are not limited to, cognitive and language deficits, apraxias, depression, delusions and other neuropsychiatric symptoms and signs, and movement and gait abnormalities) which comprises administering to the subject a therapeutically effective amount of a compound to inhibit the binding of an amyloid beta peptide

30 (preferably,  $A\beta_{1-42}$ ) with nAChRs, preferable alpha-7 nAChRs, most preferably, human alpha-7 nAChRs.

In a second embodiment of the invention is a method of improving memory and/or mental status and/or of halting the progression of mental deterioration in an Alzheimer's disease patient which comprises administering to the subject a therapeutically effective amount of a compound to inhibit the binding of an amyloid beta peptide (preferably,  $A\beta_{1-42}$ ) with nAChRs, preferably alpha-7 nAChRs, most preferably, human alpha-7 nAChRs.

Preferably, the compound used in the methods of treating neurodegenerative disorders, treating and/or preventing Alzheimer's disease, and improving memory and/or halting the progression of mental deterioration in an Alzheimer's disease patient is not estrogen, raloxifene, droloxifene, tamoxifen, idoxifene or levomeloxifene; more preferably, the compound is not estrogen or a selective estrogen receptor modulator (SERM). A SERM is an estrogen receptor ligand that exhibits estrogen agonist activity in the cardiovascular system, CNS and bone, and estrogen antagonist activity in reproductive tissues, such as breast and uterus.

Also included in the invention is the use of a compound which inhibits the binding of an amyloid beta peptide (preferably  $A\beta_{1-42}$ ) to an alpha-7 nAChR (preferably, a human alpha-7 nAChR) in the preparation of a medicament for the treatment of a neurodegenerative disorder in a subject (preferably, a human) in need thereof.

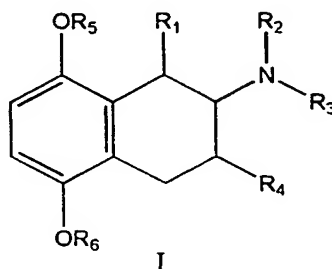
Another illustration of the invention is the use of a compound which inhibits the binding of an amyloid beta peptide (preferably  $A\beta_{1-42}$ ) to alpha-7 nAChRs (preferably, human alpha-7 nAChRs) in the preparation of a medicament for: a) improving memory, b) halting the progression of the mental deterioration seen in Alzheimer's disease patients, c) treating dementia, d) preventing dementia in an Alzheimer's patient, and e) treating and/or preventing other clinical manifestations of Alzheimer's disease that include, but are not limited to, cognitive and language deficits, apraxias, depression, delusions and other



neuropsychiatric symptoms and signs, and movement and gait abnormalities in an Alzheimer's patient.

In another aspect of the invention is a compound of the formula I:

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wherein R<sub>1</sub> is hydrogen or C<sub>1</sub>-C<sub>4</sub> alkyl;

R<sub>2</sub> is selected from hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, aryl or C<sub>7</sub>-C<sub>10</sub> aralkyl;

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R<sub>3</sub> is selected from hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>3</sub>-C<sub>10</sub> alkenyl,

C<sub>3</sub>-C<sub>8</sub> cycloalkyl, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy carbonyl, C<sub>1</sub>-C<sub>6</sub> alkyl,

C<sub>1</sub>-C<sub>6</sub> alkylthio, heteroaryl, C<sub>1</sub>-C<sub>4</sub> alkyl, unsubstituted or substituted aryl or unsubstituted or substituted C<sub>7</sub>-C<sub>10</sub> aralkyl wherein the substituent on the aryl or aralkyl are one or more substituents independently selected from the group

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consisting of halogen, hydroxy, C<sub>1</sub>-C<sub>6</sub> alkyl and unsubstituted or substituted

C<sub>1</sub>-C<sub>6</sub> alkoxy wherein the substituents on the alkoxy are one or more substituents independently selected from amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>1</sub>-C<sub>6</sub> dialkylamino, pyrrolidinyl, piperidinyl, azepinyl or morpholinyl; or

R<sub>2</sub> and R<sub>3</sub>, together with the nitrogen to which they are attached, form a five or six-membered heterocyclic ring selected from pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl or piperazinyl;

R<sub>4</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, or C<sub>7</sub>-C<sub>10</sub> aralkyl; and

R<sub>5</sub> and R<sub>6</sub> are each independently selected from hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>3</sub>-C<sub>10</sub> alkenyl, C<sub>1</sub>-C<sub>8</sub> alkyl carbonyl, or diphenylphosphinyl; and pharmaceutically acceptable salts and prodrugs thereof.

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In preferred compounds of formula I,

R<sub>1</sub> is hydrogen;

$R_2$  is selected from hydrogen or  $C_1$ - $C_4$  alkyl;

$R_3$  is selected from  $C_1$ - $C_4$  alkyl,  $C_3$ - $C_{10}$  alkenyl,

$C_5$ - $C_6$  cycloalkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_4$  alkyl,

$C_1$ - $C_6$  alkylthio, heteroaryl,  $C_1$ - $C_4$  alkyl, or unsubstituted or substituted

- 5  $C_7$ - $C_{10}$  aralkyl wherein the substituent on the aralkyl are one or two substituents independently selected from the group consisting of halogen, hydroxy,  $C_1$ - $C_4$  alkyl and unsubstituted or substituted  $C_1$ - $C_4$  alkoxy wherein the substituents on the alkoxy are one or two substituents independently selected from amino,  $C_1$ - $C_4$  alkylamino,  $C_1$ - $C_4$  dialkylamino, pyrrolidinyl, or piperidinyl; or

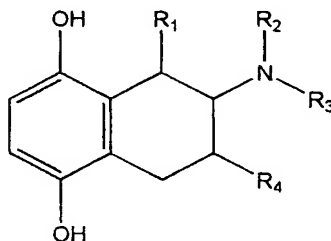
- 10  $R_2$  and  $R_3$ , together with the nitrogen to which they are attached, form a morpholinyl ring;

$R_4$  is  $C_1$ - $C_4$  alkyl; and

$R_5$  and  $R_6$  are each independently selected from hydrogen,  $C_1$ - $C_4$  alkyl,  $C_3$ - $C_6$  alkenyl,  $C_1$ - $C_6$  alkylcarbonyl, or diphenylphosphinyl.

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In a subclass of compounds of formula I are compounds having the formula



- 20 wherein  $R_1$  is hydrogen or  $C_1$ - $C_4$  alkyl;

$R_2$  and  $R_3$  are each independently selected from hydrogen,  $C_1$ - $C_6$  alkyl, aryl or  $C_7$ - $C_{10}$  aralkyl; and

$R_4$  is  $C_1$ - $C_6$  alkyl, aryl, or  $C_7$ - $C_{10}$  aralkyl;

and pharmaceutically acceptable salts thereof.

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Compounds of formula I are novel compounds which block the interaction of beta-amyloid with alpha-7 nAChRs. More specifically, the compounds of formula I inhibit the binding of  $A\beta_{1-42}$  with human alpha-7

nAChRs by binding to A $\beta$ <sub>1-42</sub>. The orientation between the nitrogen atom and R<sub>4</sub> on the appropriate ring can be either *cis* or *trans*. Preferably, the compound is 5,8-dihydroxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene, and pharmaceutically acceptable salts thereof.

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Other compounds useful in the methods of the present invention inhibit the binding of A $\beta$ <sub>1-42</sub> with human alpha-7 nAChRs by binding to human alpha-7 nAChRs directly. An example of such a compound which binds to human alpha-7 nAChRs is  $\alpha$ -bungarotoxin.

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#### Detailed Description of the Invention

The present invention provides methods of treating neurodegenerative disorders by inhibiting the binding of amyloid beta peptides to alpha-7 nAChRs.

15 Neurodegenerative disorders included within the methods of the present invention include, but are not limited to, Alzheimer's disease, Pick's disease, diffuse Lewy body disease, progressive supranuclear palsy (Steel-Richardson syndrome), multisystem degeneration (Shy-Drager syndrome), motor neuron diseases including amyotrophic lateral sclerosis, degenerative ataxias, cortical

20 basal degeneration, ALS–Parkinson's–Dementia complex of Guam, subacute sclerosing panencephalitis, Huntington's disease, Parkinson's disease, synucleinopathies, primary progressive aphasia, striatonigral degeneration, Machado-Joseph disease/spinocerebellar ataxia type 3 and olivopontocerebellar degenerations, Gilles De La Tourette's disease, bulbar

25 and pseudobulbar palsy, spinal and spinobulbar muscular atrophy (Kennedy's disease), primary lateral sclerosis, familial spastic paraplegia, Werdnig-Hoffmann disease, Kugelberg-Welander disease, Tay-Sach's disease, Sandhoff disease, familial spastic disease, Wohlfart-Kugelberg-Welander disease, spastic paraparesis, progressive multifocal leukoencephalopathy, and

30 prion diseases (including Creutzfeldt-Jakob, Gerstmann-Sträussler-Scheinker disease, Kuru and fatal familial insomnia).

Other conditions also included within the methods of the present invention include age-related dementia and other dementias and conditions with memory loss including vascular dementia, diffuse white matter disease (Binswanger's disease), dementia of endocrine or metabolic origin, dementia of head trauma and  
5 diffuse brain damage, dementia pugilistica and frontal lobe dementia. Also other neurodegenerative disorders resulting from cerebral ischemia or infarction including embolic occlusion and thrombotic occlusion as well as intracranial hemorrhage of any type (including, but not limited to, epidural, subdural, subarachnoid and intracerebral), and intracranial and intravertebral lesions  
10 (including, but not limited to, contusion, penetration, shear, compression and laceration).

Preferably, the neurodegenerative disorder is selected from Alzheimer's disease, Parkinson's disease, Tourette's syndrome, amyotrophic lateral sclerosis,  
15 age-related memory loss, senility and age-related dementia, most preferably, the neurodegenerative disorder is Alzheimer's disease. Because, most preferably, the neurodegenerative disorder is Alzheimer's disease, also defined as an amyloidosis, other conditions within the methods of the present invention include other amyloidosis which share features including, but not limited to, hereditary  
20 cerebral angiopathy, nonneuropathic hereditary amyloid, Down's syndrome, macroglobulinemia, secondary familial Mediterranean fever, Muckle-Wells syndrome, multiple myeloma, pancreatic- and cardiac-related amyloidosis, chronic hemodialysis arthropathy, and Finnish and Iowa amyloidosis.

25 The terms "amyloid beta", "amyloid beta peptide" or "beta-amyloid" as used herein, refer to amyloid beta peptides and include the  $A\beta_{1-40}$ ,  $A\beta_{1-42}$  and  $A\beta_{1-43}$  peptides and their fragments. Examples of fragments of amyloid beta peptides that have been shown to have biological activity and are useful in the methods of the present invention include, but not limited to, fragment 1-28, and fragment  
30 25-35 (e.g., Yatin SM, Aksenov M, Butterfield DA. Neurochem Res 1999 Mar;24(3):427-35; Hirakura Y, Satoh Y, Hirashima N, Suzuki T, Kagan BL, Kirino Y. Biochem Mol Biol Int 1998 Nov;46(4):787-94; Mazziotti M, Perlmutter

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- 20           The term "subject" as used herein, refers to an animal, preferably a mammal, most preferably a human, who has been the object of treatment, observation or experiment.

- 25           The term "therapeutically effective amount" as used herein, means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated.

- 30           The term "alkyl" shall mean straight or branched chain alkanes of one to ten carbon atoms, or any number within this range. For example, alkyl radicals include, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl,

n-pentyl, 3-(2-methyl)butyl, 2-pentyl, 2-methylbutyl, neopentyl, n-hexyl, 2-hexyl and 2-methylpentyl. Alkoxy radicals are oxygen ethers formed from the previously described straight or branched chain alkyl groups. Cycloalkyl groups contain 3 to 8 ring carbons and preferably 5 to 7 carbons. Similarly,  
5 alkenyl and alkynyl groups include straight and branched chain alkenes and alkynes having 2 to 10 carbon atoms, or any number within this range.

The term "aryl" indicates aromatic groups such as phenyl and naphthyl.

10 The term "C<sub>7</sub>-C<sub>10</sub> aralkyl" means an alkyl group substituted with an aryl group wherein the total number of carbon atoms is between 7 and 10 (e.g., benzyl, phenylethyl, phenylpropyl).

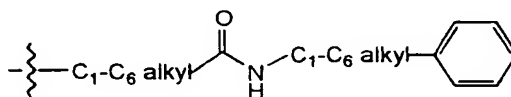
The term "heteroaryl" as used herein represents an unsubstituted or  
15 substituted stable five or six membered monocyclic aromatic ring system or an unsubstituted or substituted nine or ten membered benzo-fused heteroaromatic ring system or bicyclic heteroaromatic ring system which consists of carbon atoms and from one to four heteroatoms selected from N, O or S, and wherein the nitrogen or sulfur heteroatoms may optionally be oxidized, and the nitrogen  
20 heteroatom may optionally be quaternized. The heteroaryl group may be attached at any heteroatom or carbon atom that results in the creation of a stable structure. Examples of heteroaryl groups include, but are not limited to pyridyl, pyridazinyl, thienyl, furanyl, imidazolyl, isoxazolyl, oxazolyl, pyrazolyl, pyrrolyl, thiazolyl, thiadiazolyl, triazolyl, benzimidazolyl, benzofuranyl,  
25 benzothienyl, benzisoxazolyl, benzoxazolyl, benzopyrazolyl, indolyl, benzothiazolyl, benzothiadiazolyl, benzotriazolyl adeninyl or quinolinyl. Preferred heteroaryl groups include pyridyl, pyrrolyl, pyrazinyl, thiadiazolyl, pyrazolyl, thienyl, triazolyl and quinolinyl.

30 The term "N(CH<sub>2</sub>)<sub>5</sub>" means a piperidinyl group.

The term "cC<sub>6</sub>H<sub>11</sub>" refers to a cyclohexyl group.

When a particular group is "substituted" (e.g., aryl, aralkyl), that group may have one or more substituents, preferably from one to five substituents, more preferably from one to three substituents, most preferably from one to  
 5 two substituents, independently selected from the list of substituents.

Under standard nomenclature used throughout this disclosure, the terminal portion of the designated side chain is described first, followed by the adjacent functionality toward the point of attachment. Thus, for example, a "phenylC<sub>1</sub>-C<sub>6</sub>  
 10 alkylamidoC<sub>1</sub>-C<sub>6</sub>alkyl" substituent refers to a group of the formula



Compounds which are useful in the methods of the present invention for  
 15 inhibiting the interaction of A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> to the alpha-7 subtype of nAChRs for either the purpose of direct therapeutic intervention or in order to screen for compounds which act via this mechanism include compounds of formula I, especially 5,8-dihydroxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (Compound 9), (-)-nicotine, (*rac*)-epibatidine,  $\alpha$ -  
 20 bungarotoxin, and pharmaceutically acceptable salts thereof.

Certain peptide stretches of the human alpha-7 nAChR bind to amyloid beta and can be used in place of the alpha-7 nAChR together with amyloid beta for the purpose of screening libraries to find compounds which block the  
 25 interaction of amyloid beta and the human alpha-7 nAChR. Included among these peptide stretches of the human alpha-7 nAChR are alpha-7 nAChR193-224 and smaller peptides derived thereof as listed in the Table.

Table

Compound	Amelioration of the A $\beta$ <sub>1-42</sub> Meditated Inhibition of ACh Release in Rat Cortical Synaptosomes (%)
human alpha-7 nAChR193-224 Ac-NGEWDLVGIPGKRSERFYECCKEPYDPDVTFTV-NH2	81% at 10 $\mu$ M 79% at 1 $\mu$ M
human alpha-7 nAChR200-214 Ac-GIPGKRSERFYECCCK-NH2	79% at 10 $\mu$ M 71% at 1 $\mu$ M
human alpha-7 nAChR206-216 Ac-SERFYECCKEP-NH2	81% at 10 $\mu$ M 77% at 1 $\mu$ M
human alpha-7 nAChR206-216 oxidized (cyclic) CC: Ac-SERFYECCKEP-NH2	84% at 10 $\mu$ M 82% at 1 $\mu$ M
human alpha-7 nAChR206-216 SERFYECCKEP	22% at 10 $\mu$ M 10% at 1 $\mu$ M
human alpha-7 nAChR210-213 Ac-YECC-NH2	72% at 10 $\mu$ M 59% at 1 $\mu$ M

The standard one-letter code for the amino acids has been employed for the compounds. This code is listed in Lehninger, A. I. "Biochemistry" Second Edition, Worth Publishers, Inc., New York, 1976, p 73-75.

For use in medicine, the salts of the compounds of this invention refer to non-toxic "pharmaceutically acceptable salts." Other salts may, however, be useful in the preparation of compounds according to this invention or of their pharmaceutically acceptable salts. Suitable pharmaceutically acceptable salts of the compounds of this invention include acid addition salts which may, for example, be formed by mixing a solution of the compound with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, citric acid,

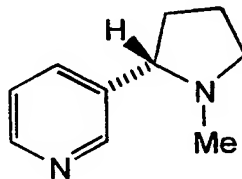


tartaric acid, carbonic acid or phosphoric acid. Furthermore, where the compounds of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may include alkali metal salts, e.g., sodium or potassium salts; alkaline earth metal salts, e.g., calcium or magnesium salts; 5 and salts formed with suitable organic ligands, e.g., quaternary ammonium salts.

The present invention includes within its scope prodrugs of the compounds of this invention. A prodrug is inactive as administered, but 10 becomes activated *in vivo*. The prodrug is converted to the parent drug chemically or by specific enzyme(s). Higuchi, T.; Stella, V., Eds. "Pro-Drugs as Novel Drug Delivery Systems"; American Chemical Society: Washington, DC, 1976. In general, such prodrugs will be functional derivatives of the compounds which are readily convertible *in vivo* into the required compound. 15 Thus, in the methods of treatment of the present invention, the term "administering" shall encompass the treatment of the various disorders described with the compound specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound *in vivo* after administration to the patient. Conventional procedures 20 for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", ed. H. Bundgaard, Elsevier, 1985.

Where the compounds according to this invention have at least one chiral center, they may accordingly exist as enantiomers. Where the 25 compounds possess two or more chiral centers, they may additionally exist as diastereomers. It is to be understood that all such isomers and mixtures thereof are encompassed within the scope of the present invention. Furthermore, some of the crystalline forms for the compounds may exist as polymorphs and as such are intended to be included in the present invention. 30 In addition, some of the compounds may form solvates with water (i.e., hydrates) or common organic solvents, and such solvates are also intended to be encompassed within the scope of this invention.

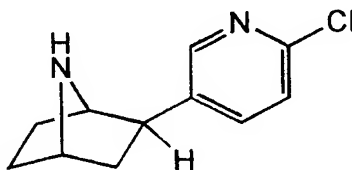
(-)-Nicotine is (-)-1-methyl-2-(3-pyridinyl)pyrrolidine and is readily available from Sigma Chemical Company.



(-)-Nicotine

5

(+/-)-Epibatidine is exo-(+/-)-2-(6-chloro-3-pyridinyl)-7-azabicyclo[2.2.1]heptane and is readily available from Sigma Chemical Company.



(rac)-Epibatidine

10

$\alpha$ -Bungarotoxin is a 74 amino acid peptide which is commercially available from Research Biochemicals Inc.  $\alpha$ -Bungarotoxin and its amino acid sequence are described in Lee, C. Y. *Annu. Rev. Pharmacol.* **1972**, 12, 265-281.

15

$^{125}\text{I}$ -A $\beta_{1-40}$ , fluo-A $\beta_{1-40}$ , and anti-alpha-7 nAChR antibodies are commercially available Amersham Pharmacia Biotech, Advanced Bioconcepts and Research Biochemicals International, respectively.

20

$^{125}\text{I}$ - $\alpha$ -bungarotoxin is commercially available from Amersham Pharmacia Biotech.

The present invention therefore provides a method of treating a neurodegenerative disorder, which comprises administering any of the compounds as defined herein in a quantity effective to treat the neurodegenerative disorder. Preferably, the compound is not estrogen, raloxifene, droloxifene, tamoxifen, idoxifene or levomeloxifene; more preferably, the compound is not estrogen or a selective estrogen receptor modulator (SERM). The compound may be administered to a patient afflicted with a neurodegenerative disorder by any conventional route of administration, including, but not limited to, intravenous, oral, subcutaneous, intramuscular, intradermal, buccal, intracerebral and other parenteral routes. The quantity of the compound which is effective for treating a neurodegenerative disorder is between 0.01 mg per kg and 10 mg per kg of subject body weight.

The method of treating neurodegenerative disorders described in the present invention may also be carried out using a pharmaceutical composition comprising any of the compounds as defined herein and a pharmaceutically acceptable carrier. The pharmaceutical composition may contain between about 0.5 mg and 200 mg of the compound, and may be constituted into any form suitable for the mode of administration selected. Carriers include necessary and inert pharmaceutical excipients, including, but not limited to, binders, suspending agents, lubricants, flavorants, sweeteners, preservatives, dyes, and coatings. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, caplets, and powders, and liquid forms, such as solutions, syrups, elixers, and suspensions. Forms useful for intracerebral and other parenteral routes of administration include sterile solutions, emulsions and suspensions.

Optimal dosages to be administered may be readily determined by those skilled in the art, and will vary with the particular compound used, the mode of administration, the strength of the preparation, the mode of administration, and the advancement of the disease condition. In addition, factors associated with the particular patient being treated, including patient age, body weight, diet, physical

activity and time of administration, and associated co-morbidities and clinical conditions will result in the need to adjust dosages.

The present invention also provides diagnostic tools useful for diagnosing Alzheimer's disease. Alzheimer's disease (AD) exhibits neuropathological abnormalities in the olfactory system located in the nasal cavity. These include the presence of dystrophic neurites that exhibit immunoreactivity for tau, neurofilaments, apolipoprotein E and other proteins, abnormal tau protein, increase in superoxide dismutase, and beta-amyloid deposition in the primary sensory (olfactory receptor) cells and nerve fibres of the nasal mucosa tissue (Arnold et al., Ann N Y Acad Sci 1998 Nov 30;855:762-75; Hock et al., Eur Neurol 1998 Jul;40(1):31-6; Johnson et al., Neurobiol Aging 1994 Nov-Dec;15(6):675-80; Kulkarni-Narla et al., Exp Neurol 1996 Aug;140(2):115-25; Lee et al., Exp Neurol 1993 May;121(1):93-105; Tabaton et al., Neurology 1991 Mar;41(3):391-4; Talamo et al., Ann N Y Acad Sci 1991;640:1-7; Yamagishi et al., Ann Otol Rhinol Laryngol 1998 May;107(5 Pt 1):421-6; Yamagishi et al., Nippon Jibiinkoka Gakkai Kaiho 1994 Jan;97(1):51-60). These observations recapitulate the neuropathological profile and neurodegenerative abnormalities (e.g., cytoskeletal changes, protein immunoreactivity and beta-amyloid deposition) observed in central nervous system neurons from AD patients. Routine access to these sensory neurons and fibers can be done with nasal biopsy in AD patients (e.g., Feron et al., Arch Otolaryngol Head Neck Surg 1998 Aug;124(8):861-6).

Olfactory neuroblasts (olfactory neurons obtained by biopsy and placed in primary cell culture) from AD patients produce carboxy terminal amyloid precursor protein (APP) fragments that contain beta-amyloid (A-beta)(Crino et al., Ann Otol Rhinol Laryngol 1995 Aug;104(8):655-61). Crino et al. showed labeling of A-beta in the basal third of the olfactory neuroepithelium and in axons projecting through the lamina propria of AD patients. Thioflavin-S staining that detects amyloid deposition was also observed in the basal third of the olfactory neuroepithelium from AD patients. Alpha 7 nicotinic acetylcholine

receptors are present in olfactory neurons probably including olfactory receptor cells in the nasal cavity (Alkondon et al., *Neurosci Lett* 1994 Aug 1;176(2):152-6; Alkondon et al., *Eur J Neurosci* 1997 Dec;9(12):2734-42; Bouvet et al., *Neurosci Res* 1988 Feb;5(3):214-23; Edwards et al., *Experientia* 1987 Aug 15;43(8):868-73; Edwards et al., *Experientia* 1988 Mar 15;44(3):208-11; Seguela et al., *J Neurosci* 1993 Feb;13(2):596-604).

Beta-amyloid peptide increases cytosolic-free  $\text{Ca}^{2+}$  in AD lymphoblasts (Ibarreta et al., *Alzheimer Dis Assoc Disord* 1997 Dec;11(4):220-7), and elevates mitogen-induced  $\text{Ca}^{2+}$  responses in freshly prepared human lymphocytes (Eckert et al., *Life Sci* 1994;55(25-26):2019-29). Amyloid precursor protein (APP) can be induced on the cell surface of human lymphocytes upon stimulation (Bullido et al., *Biochim Biophys Acta* 1996 Aug 21;1313(1):54-62) and increased APP-770 isoform occurs in lymphocytes from AD patients (Ebstein et al., *Brain Res Mol Brain Res* 1996 Jan;35(1-2):260-8). Lymphoblastoid cells from patients with early-onset and late-onset familial AD show increased expression of beta-APP mRNA and protein (Matsumoto et al., *Eur J Biochem* 1993 Oct 1;217(1):21-7). Lymphocytes from AD patients also exhibit an increased mRNA level for alpha 7 nicotinic receptor (Hellstrom-Lindahl et al., *Brain Res Mol Brain Res* 1999 Mar 20;66(1-2):94-103)

Based on the information described above, we propose that the analysis of the alpha 7 nicotinic acetylcholine receptor – beta amyloid peptides interaction in circulating blood cells and olfactory neuroepithelial neurons/neuronal processes or olfactory neuroblasts obtained from AD patients could be used as AD diagnostic tools, markers of AD progression and prognosis, and markers of therapeutic efficacy for any intervention or treatment targeting AD.

Thus, the present invention provides methods for diagnosing Alzheimer's disease, monitoring the progression and prognosis of Alzheimer's

disease and/or monitoring the therapeutic efficacy of any intervention or treatment of Alzheimer's disease comprising:

- (a) obtaining a test sample from a subject wherein the test sample comprises circulating blood cells and/or olfactory neuroepithelial neuronal cell bodies or their neuronal processes (i.e., dendrites and axon of a neuron); and
- (b) analyzing the test sample for interaction of an amyloid beta peptide (including, but not limited to, A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>1-42</sub> and A $\beta$ <sub>1-43</sub> peptides and their fragments) with alpha-7 nicotinic acetylcholine receptors.

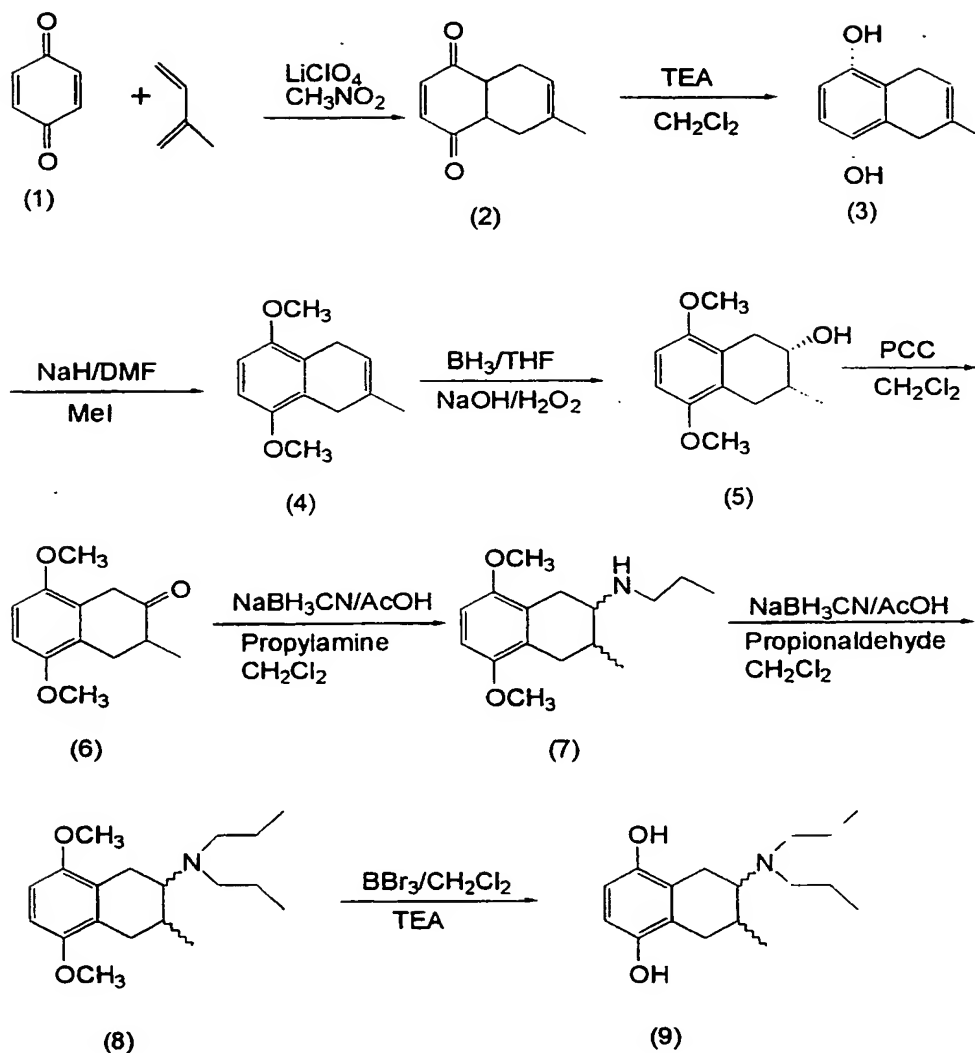
The compounds of formula I, such as 5,8-Dihydroxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (Compound 9), are made according to the procedures described in the Schemes and Examples which follow.

Abbreviations used in the instant specification, particularly the Schemes and Examples, are as follows:

	Ac	=	acetyl
	Ach	=	acetylcholine
20	AcOH	=	acetic acid
	BSA	=	bovine serum albumin
	DMF	=	N,N-dimethyl formamide
	DMSO	=	dimethyl sulfoxide
	Et <sub>3</sub> N	=	triethyl amine
25	EtOAc	=	ethyl acetate
	FCS	=	fetal calf serum
	i-Pr	=	isopropyl
	Me	=	methyl
	Mel	=	methyl iodide
30	nAChR	=	nicotinic acetylcholine receptor
	Ph	=	phenyl
	PCC	=	pyridinium chlorochromate

TEA = triethyl amine  
 THF = tetrahydrofuran  
 TLC = thin layer chromatography

## SCHEME 1

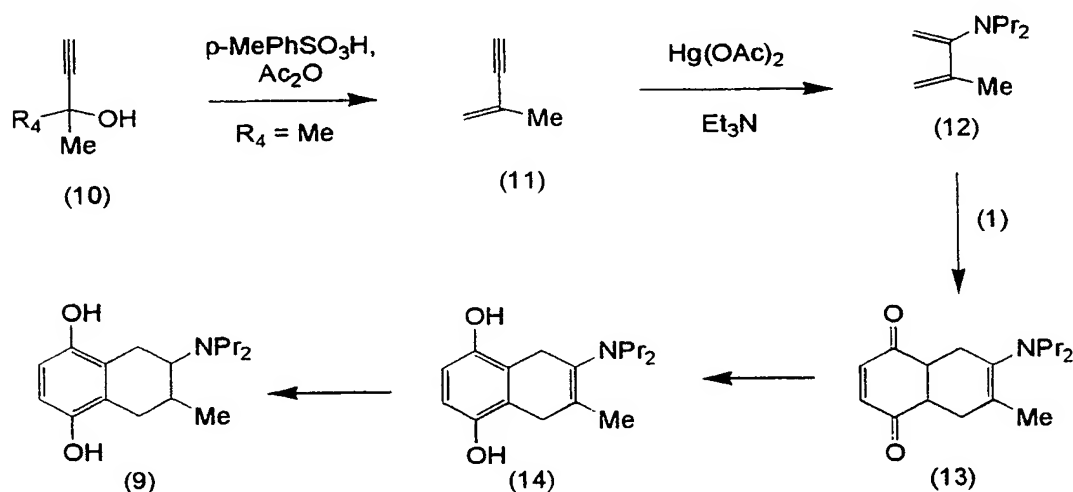


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Compounds of the invention can be prepared as shown in Scheme 1. First a Diels-Alder reaction is performed on benzophenone (1) with a suitable diene such as isoprene to give a dione such as (2). Then, base-catalyzed isomerization of (2) affords a 1,4-dihydroxyphenyl compound (3), which is converted to dimethyl ether (4). Hydroboration followed by oxidation give alcohol (5), which is then oxidized to give ketone (6). Reductive amination with an amine such as propyl

amine is then carried out using a suitable hydride reducing agent such as sodium cyanoborohydride, to give, for example, compound 7. This material can then be subjected to a reductive amination reaction such as with propionaldehyde as shown to yield (8). Compound (8) is then treated with HBr in acetic acid to cleave the methyl ethers and form dihydroxy compound (9). Alternatively, BBr<sub>3</sub> may be used to cleave the methyl ethers and form dihydroxy compound (9). Moreover, a deficiency of BBr<sub>3</sub> may be used to provide compounds in which only one of the methyl ethers has been removed to afford compounds with one hydroxy and one methoxy group.

10



Scheme 2

A further means of preparing compounds of the present invention is illustrated in Scheme 2. A suitable propargyl alcohol, such as 3-hydroxy-3-methyl-1-butyne (10, R<sub>4</sub> = Me) is dehydrated to give an enyne such as compound 11. This material is then subjected to an aminomercuriation reaction to afford a 2-amino-1,4-butadiene such as compound (12). Diels-Alder reaction of compound (12) with benzophenone (1) gives (13) which can be isomerized as in Scheme 1 with base to give the 1,5-dihydroxy compound (14). Reduction of the double bond of (14) catalytically with hydrogen and palladium on carbon, such as 10% palladium on carbon, yields compounds of the invention such as (15).



Compounds of type 9 can be treated with a base such as triethylamine in a suitable solvent such as dioxane or methylene chloride along with electrophiles such as acid halides, phosphinyl halides, or alkyl halides to give the products of substitution on one or both of the phenolic hydroxyls. Such compounds can be active by themselves, or serve as prodrugs for compound 9.

The following Examples are set forth to aid in the understanding of the invention, and are not intended and should not be construed to limit in any way the invention set forth in the claims which follow thereafter.

### Example 1

#### **Preparation of 5,8-Dihydroxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (9)**

##### **a. 5,8-Dihydroxy-2-methyl-1,4-dihydronaphthalene (3)**

To a solution of p-benzoquinone (10.00 g, 92.5 mmol) in 1M solution of lithium perchlorate, in nitromethane (300 mL) was added isoprene (9.24 mL, 92.3 mmol) at room temperature. The resultant reaction mixture was stirred at room temperature under N<sub>2</sub> for 4 hours. The reaction mixture was partitioned between EtOAc (500mL) and water (200 mL). The organic layer was washed with brine, dried over sodium sulfate and concentrated to give **2** as brown solid. The reaction was repeated on 15 g of benzoquinone to afford additional **2**. These two runs were combined and carried on without further purification. To a solution of **2** (35.00 g, 199 mmol) in methylene chloride (400 mL) was added triethylamine (40 mL). The reaction mixture was stirred at room temperature for 1 hr. The product was precipitated out of solution by the addition of hexane (300 mL). The solid was collected by filtration and dried in a dessicator under high vacuum overnight to provide **3** as a light brown solid.

<sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 1.75 (s, 3H), 3.00-3.01 (m, 2H), 3.09 (s, 2H), 5.53 (s, 1H), 6.43 (s, 2H), 8.49-8.52 (m, 2H).

b. 5,8-Dimethoxy-2-methyl-1,4-dihydronaphthalene (4)

To a cold (-78°C) solution of hydroquinone **3** (17.00 g, 96.6 mmol) in DMF under N<sub>2</sub> was added unwashed 60% sodium hydride in mineral oil (8.90 g, 222.5 mmol). The resultant reaction mixture was warmed to room temperature over a 30 min period and subsequently treated with methyl iodide (14.3 mL, 230 mmol). After stirring the reaction mixture for 1 hr at room temperature the reaction mixture was diluted with ethyl acetate (1.5 L) and washed with brine (3X500ml). The organic solution was dried over sodium sulfate, filtered, concentrated, and the residue purified on silica gel (elution with 20% ethyl acetate/hexane) to afford **4** as a light yellow oil which solidified upon standing.

<sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ 1.77 (s, CH<sub>3</sub>), 3.07-3.09 (m, 2H), 3.16 (br s, 2H), 3.73(s, 3H), 3.75 (s, 3H), 5.52-5.53 (m, 1H), 6.65 (s, 2H).

c. 5,8-Dimethoxy-trans-2-hydroxy-3-methyl-1,2,3,4-tetrahydronaphthalene (5)

A solution of **4** (5.00 g, 24.5 mmol) in tetrahydrofuran (100ml) was cooled to 0 °C and treated with 1 M borane-tetrahydrofuran complex (24.5mL, 24.5mmol) and the resulting solution was stirred at room temperature for 4 h. Then, 12mL of 3 N NaOH solution was added slowly to the stirring reaction solution followed by the addition of 6 mL of 30% aqueous hydrogen peroxide. After stirring for 30 min, the resultant mixture was diluted with 500 mL of ethyl acetate and washed by brine (2X100mL). The organic layer was dried over sodium sulfate, filtered and concentrated, and the residue was purified on silica gel (30% ethyl acetate/hexane) to give **5** as a white solid.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.13 (d, 3H, J=6.51 Hz), 1.60 (d, 1H), 1.75-1.90 (m, 1H), 2.26 (dd, 1H, J=10.0 Hz, 17.57 Hz), 2.48 (dd, 1H, J=8.93 Hz, 17.10 Hz), 2.96 (dd, 1H, J=5.31,17.7Hz), 3.16 (dd, 1H, J=5.33, 5.35 Hz), 3.63-3.69 (m, 1H), 3.78 (s, 6H), 6.62 (s, 2H).

d. 5,8-Dimethoxy-3-methyl-2-tetralone (6)

A solution of alcohol **5** (10 g, 45 mmol) and dichloromethane (200 mL) at -30°C was treated dropwise with oxalyl chloride (4.92 g, 24 mL, 0.275 mol). The reaction was stirred at -30°C for 30 min, cooled to -60°C, and DMSO (6.4 mL) was added slowly over 15 min. The reaction was stirred for one hour,  
5 cooled to -78°C for 30 min, and then treated dropwise with triethylamine (40 mL). The reaction was warmed to room temperature and stirred for 1 hr, followed by the addition of water and dichloromethane with thorough mixing. The organic layer was separated, dried with MgSO<sub>4</sub>, filtered and the solvent was evaporated to an oil. Purification of this material using flash  
10 chromatography (flash silica, 70/30: hexane/EtOAc) afforded **6** as a white crystalline solid.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.7 (q, 2 H), 3.78 (s, 3 H), 3.75 (s, 3 H), 3.5 (dd, 2 H), 3.3 (m, 1 H), 2.55 (m, 2 H), 1.2 (d, 3 H).

15 e. 5,8-Dimethoxy-2-*N*-propylamino-3-methyl-1,2,3,4-tetrahydronaphthalene (7)

To a solution of **6** (0.15 g, 0.68 mmol) in acetonitrile (10 mL) was added sodium cyanoborohydride (0.085 g, 1.30 mmol), 0.05 mL of acetic acid and *N*-propylamine (0.08 mL, 1.3 mmol). The resulting solution was stirred at room  
20 temperature overnight and poured into 10 mL of 1N NaOH solution. The mixture was extracted with EtOAc, dried over sodium sulfate, concentrated, and the residue purified by preparative TLC (elution with 30% ethyl acetate in hexane) to give amine **7** as a colorless oil.

<sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ 0.85 (d, 3H, J= 7.07 Hz), 0.93-0.97 (m, 3H), 1.09 (t, 2H), 1.49-1.61 (m, 2H), 2.12-2.42 (m, 2H), 2.51-2.76 (m, 4H),  
25 2.82-3.08 (m, 2H), 3.73 (s, 3H), 3.74 (s, 3H), 6.65 (s, 2H).

f. 5,8-Dimethoxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (8)

30 To a solution of **7** (0.11g, 0.42 mmol) in acetonitrile (10 mL) was added sodium cyanoborohydride (0.05 gm, 0.6 mmol), 0.03 mL of acetic acid and propionaldehyde (0.045 mL, 0.60 mmol). The resulting solution was stirred at

room temperature overnight and poured into 10 mL of 1N NaOH solution. The mixture was extracted with EtOAc, dried over sodium sulfate, concentrated, and the residue was purified by preparative TLC (elution with 30% ethyl acetate in hexane) to give amine **8** as a light yellow solid.

- 5    <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ 0.84 (d, 3H, J= 6.94 Hz), 0.92 (t, 2H, J= 7.30, 7.32Hz), 1.27-1.59 (m, 4H), 2.33-2.42 (m, 2H), 2.54-3.02 (m, 8H), 3.73 (s, 3H), 3.76 (s, 3H), 6.66 (s, 2H).

In a similar manner was prepared compounds **19**, **21**, **25**, **27**, **29**, **35**, **37**, **39**, **41**, **52**, and **54**.

- 10        In order to separate the enantiomers of **8**, 2.859 g of **8** was passed through a CHIRALPAK® AD™ chiral cellulose-based high pressure liquid chromatography column (8 cm X 30 cm) at 25° C using hexane/isopropyl alcohol (99/1) as the eluant to give two fractions as oils. Fraction 1 (**18**) had a 98.6% enantiomeric excess (e.e.) and fraction 2 (**17**) had a 97.9% e.e.
- 15    Fraction 1 (0.306g, 1.0 mmol) and fumaric acid (0.141 g, 1.2 mmol) were dissolved in ethanol (2 mL) with heating. The ethanol was evaporated and the residue triturated with diethyl ether affording the fumarate salt of **18** as a white solid. Similarly obtained from Fraction 2 (0.301g, 1.0 mmol) and fumaric acid (0.141 g, 1.2 mmol) was the fumarate sale of **17**.

20

g.    5,8-Dihydroxy-trans-2-di(N-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (9)

- A mixture of **8** (1.4 g, 4.6 mmol) and 3.5 mL of 48 % aqueous HBr in acetic acid (3.5 mL) was stirred at 100°C under N<sub>2</sub> overnight. The reaction mixture was then cooled to 0°C and extracted with diethyl ether (20 mL). The organic solution was neutralized with aqueous NaHCO<sub>3</sub> and separated. The aqueous layer was extracted with ether (20 mL) and the combined ether solutions were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated to give **9**.
- 25

- <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 0.86-0.92 (dt, 6H), 1.13 (d, 3H, J=6.42 Hz), 1.42-1.50 (m, 4H), 1.88-1.93 (m, 1H), 2.23-2.28 (m, 1H), 2.39-2.52 (m, 6H), 2.61-2.63 (m, 1H), 2.90 (dd, 1H, J=4.75, 16.28 Hz), 2.95 (dd, 1H, J=5.05,
- 30

16.75Hz), 4.84 (br s, 2H), 6.50 (dd, 2H, J=8.52Hz). CI-MS: m/e MH<sup>+</sup> 278 (40%).

In a similar manner **17** and **18** were converted to **15** and **16** respectively. Additionally, demethylation of the appropriate dimethyl ethers by the procedure described here led to the preparation of compounds **20**, **22**, **26**, **28**, **30**, **32**, **34**, **36**, **40**, **42**, **53**, and **55**. In the course of the demethylation of **37**, the butyl ether was also removed to obtain **38**.

### Example 2

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#### **Preparation of 5,8-Dihydroxy-*cis*-2-(1-morpholinyl)-3-methyl-1,2,3,4-tetrahydronaphthalene (24)**

##### a. 5,8-Dimethoxy-*cis*-2-(1-morpholinyl)-3-methyl-1,2,3,4-tetrahydronaphthalene (23)

15

Ketone **6** (0.299 g, 1.36 mmol) was dissolved in toluene (1 mL) followed by the addition of morpholine (0.130 g, 0.13 mL, 1.5 mmol) and Ti(*i*-PrO)<sub>4</sub> (0.611 g, 0.64 mL, 2.15 mmol). The reaction mixture was stirred for 15 hrs at room temperature. Methanol (2 mL) was added followed by portionwise addition of NaBH<sub>4</sub> (0.15 g, 3.9 mmol) over 1 hr. Dichloromethane and 1N NaOH were added with thorough mixing and the organic layer was separated, dried with MgSO<sub>4</sub>, filtered and evaporated to an oil. Purification of this material using flash chromatography (flash silica, 75/25: hexane/EtOAc) afforded product **23**.

<sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 6.6 (s, 2 H), 3.6 (d, 6 H), 3.5 (s, 4 H), 3.2 (s, 4 H), 3.2 (s, 6 H), 2.8 (d, 1 H), 2.7 (d, 1 H), 2.3 (m, 2 H), 2.05 (m, 2 H), 0.6 (d, 3 H).

##### b. 5,8-Dihydroxy-*cis*-2-(1-morpholinyl)-3-methyl-1,2,3,4-tetrahydronaphthalene (24)

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A solution of **23** (20 mg, 0.066 mmol) and dichloromethane (2 mL) at –78°C was treated with a 1 M solution of BBr<sub>3</sub> in dichloromethane (1 mL, 1

mmol.) and stirred for 1 hr at  $-78^{\circ}\text{C}$ . After warming to room temperature, methanol (5 mL) was added and the solvents were evaporated. Methanol addition to the residue followed by evaporation was done twice. The residue was dissolved in acetonitrile (25 mL) and treated with triethylamine (2 mL).

- 5 After stirring for 2 hrs, water and ethyl acetate were added with thorough mixing. The organic layer was separated, dried with  $\text{MgSO}_4$ , filtered, and solvent evaporated to yield **24** as an oil.

$^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  6.7 (s, 2 H), 3.4 (m, 4 H), 3.3 (m, 1 H), 2.6 (m, 5 H), 1.9 (m, 1 H), 1.5 (m, 2 H), 1.05 (d, 3 H), 0.9 (m, 3 H).

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### Example 3

#### **Preparation of 5,8-Dimethoxy-*cis* and *trans*-2-(*N*-propyl-*N*-propargyl)amino-3-methyl-1,2,3,4-tetrahydronaphthalene**

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#### **(31 and 33)**

- Ketone **6** (1 g, 4.5 mmol) was dissolved in acetonitrile (75 mL) followed by the addition of acetic acid (0.54 mL, 9 mmol, 2 eq.) and propargylamine (0.6 mL, 9 mmol, 2 eq.). The reaction mixture was stirred at room temperature for 1 hr. Sodium cyanoborohydride (0.6 g, 9 mmol, 2 eq.) was added portionwise (3x) over 1.5 hrs (every 30 min), and the reaction mixture was stirred overnight at room temperature. Ethyl acetate and 1 N NaOH were added with thorough mixing and the organic layer was separated, dried with  $\text{MgSO}_4$ , filtered and evaporated to an oil. The product was purified with flash chromatography (50:50/ethyl acetate:hexane) to yield an oil of 5,8-dimethoxy-*cis* and *trans*-2-*N*-(propargyl)amino-3-methyl-1,2,3,4-tetrahydronaphthalene (1.2 g, 100%). This material was dissolved in acetonitrile along with propionaldehyde (0.65 g, 9 mmol, 2 eq.) and acetic acid (0.5 mL, 9 mmol) and stirring for 1 hr at room temperature. One hour later, sodium cyanoborohydride (0.6 g, 9 mmol, 2 eq.) was added portionwise (3x) over 1.5 hours (every 30 mins). The reaction was stirred overnight at room temperature. Ethyl acetate and 1 N NaOH were added to the reaction with thorough mixing and the organic layer was separated, dried with  $\text{MgSO}_4$ , filtered and evaporated to a mixture of products

**31 and 33.** Purification of this material using flash chromatography (flash silica, 80:20/ hexane:ether) afforded product **31** and **33**. Compound **1** H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  6.6 (s, 2 H), 3.8 (s, 6 H), 3.4 (q, 2 H), 3.0 (dd, 2 H), 2.6 (m, 4 H), 2.2 (m, 2 H), 1.85 (m, 1 H), 1.5 (m, 2 H), 1.1 (d, 3 H), 0.9 (t, 3 H).

- 5 Compound **33**: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  6.6 (s, 2 H), 3.78 (s, 3 H), 3.75 (s, 3 H), 3.6 (q, 2 H), 3.05 (dd, 1 H), 2.75 (m, 2 H), 2.61 (m, 3 H), 2.3 (m, 2 H), 2.1 (s, 1 H), 1.55 (m, 2 H), 0.9 (t, 3 H), 0.8 (d, 3 H).

#### Example 4

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**Preparation of 5-[2-(3-Methyl)butenyl]-8-hydroxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (43); and 5-Hydroxy-8-[2-(3-methyl)butenyl]-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (44)**

- 15 Diol **9** (0.3 g, 1.2 mmol) was dissolved in acetone (40 mL) followed by the addition of potassium carbonate (0.19 g) and 1-bromo-3-methyl-2-butene (0.1 mL, 0.87 mmol). The reaction mixture was stirred for 48 hrs at room temperature. Dichloromethane and water were added with thorough mixing and the organic layer was separated, dried with MgSO<sub>4</sub>, filtered and the
- 20 solvent was evaporated to yield an oil. Purification of this material using flash chromatography (flash silica, 70:30/ hexane:ether) afforded products **43** and **44**. The regiochemical structural assignment between the two of them is uncertain. Compound **43**: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  6.5 (s, 2 H), 5.5 (t, 1 H), 4.4 (m, 2 H), 3.0 (m, 2 H), 2.4 (m, 8 H), 1.8 (m, 1 H), 1.72 (s, 3 H), 1.65 (s, 3 H), 1.4 (m, 4 H), 1.1 (dd, 3 H), 0.85 (t, 6 H). Compound **44**: <sup>1</sup>H NMR (300
- 25 MHz, CDCl<sub>3</sub>)  $\delta$  6.55 (s, 2 H), 5.45 (t, 1 H), 4.4 (m, 2 H), 3.05 (dd, 1 H), 2.85 (dd, 1 H), 2.4 (m, 7 H), 1.65 (s, 3 H), 1.6 (s, 3 H), 1.55 (m, 2 H), 1.4 (m, 4 H), 1.1 (m, 4 H), 1.1 (d, 3 H), 0.9 (t, 6 H).

Example 5

**Preparation of 5-Hydroxy-8-methoxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (45); and 5-Methoxy-8-hydroxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (46)**

Compound **8** (1.85 g, 6 mmol) was dissolved in dichloromethane (20 mL) and cooled to -78°C, followed by the addition of boron tribromide (6 mL of a 1 M solution, 6 mmol, 1 eq.). The reaction mixture was stirred at -78°C for 1 hr and then warmed to room temperature for 2 hrs. The reaction was quenched with methanol (5 mL), and the solvent was evaporated (methanol quench repeated twice more). The resulting oil was dried overnight under high vacuum (5 mm Hg). The residue was dissolved in dichloromethane and NaHCO<sub>3</sub> was added, mixed thoroughly, and the organic layer was separated, dried with MgSO<sub>4</sub>, filtered and the solvent evaporated to a mixture of two regioisomers **45** and **46** (1:1). The purification of a mixture (200 mg) was conducted on a reverse phase HPLC (C18 column, 69:30:1/ water:acetonitrile:trifluoroacetic acid) to afford **45** and **46** as trifluoroacetate salts. Compound **45** (TFA salt): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.65 (q, 2H), 4.7 (bs, 1 H), 3.7 (s, 3 H), 3.5 (m, 1 H), 3.3 (m, 1H), 3.0 (m, 4H), 2.7 (m, 2 H), 2.3 (m, 1 H), 2.0 (m, 5 H), 1.15 (d, 3 H), 0.9 (m, 6 H). Compound **46** (free base): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.55 (q, 2 H), 4.3 (bs, 1 H), 3.75 (s, 3 H), 3.05 (dd, 1 H), 3.0 (dd, 1 H), 2.6 (m, 1 H), 2.4 (m, 6 H), 2.2 (m, 1 H), 1.8 (m, 1 H), 1.4 (m, 4 H), 1.1 (d, 3 H), 0.85 (t, 6 H). The regiochemistry of the structure of **46** was confirmed through careful analysis of HMBC (Heteronuclear Multiple Bond Correlation) connectivities and nOe effects. The structure of **46** was established by connectivity between the methoxy substituent on the aromatic ring and the benzylic methylene adjacent to the methyl-substituted carbon.

Example 6

**Preparation of 5,8-Dimethoxy-2-[*N*-(*L*-alanyl methyl ester)]-3-methyl-1,2,3,4-tetrahydronaphthalene (48)**



Ketone **6** (1 g, 4.5 mmol) was dissolved in acetonitrile (75 mL) and acetic acid (0.54 mL, 9 mmol, 2 eq.), and alanine methyl ester hydrochloride (1.3 g, 9 mmol, 2 eq.) was added. The reaction was stirred at room temperature for 1 hr, and then sodium cyanoborohydride (0.6 g, 9 mmol, 2 eq.) was added  
5 portionwise (3x) over 1.5 hrs (every 30 min). The reaction was stirred overnight at room temperature. Ethyl acetate and 1 N NaOH were added with thorough mixing and the organic layer was separated, dried with MgSO<sub>4</sub>, filtered and evaporated to an oil. The product was purified with flash chromatography (50:50/ ethyl acetate:hexane) to yield **48** as an oil. Compound **48**, (mixture of  
10 cis and trans stereoisomers) <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.64 (d, 2 H), 4.2 (m, 1 H), 3.85 (m, 3 H), 3.75 (s, 6 H), 3.5 (m, 1 H), 3.1 (m, 2 H), 2.8 (m, 2H), 2.55 (m, 1 H), 2.3 (m, 1 H), 1.7 (m, 3 H), 1.2 (m, 3 H).

#### Example 7

15

#### **Preparation of 5,8-Dimethoxy-2-[N-(L-alanyl methyl ester)-N-propyl]-3-methyl-1,2,3,4-tetrahydronaphthalene (47)**

Compound **48** (0.28 g, 0.8 mmol) was dissolved in acetonitrile along with propionaldehyde (0.1 mL, 1.6 mmol, 2 eq.) and acetic acid (0.2 mL, 1.6  
20 mmol), and stirred for 1 hr at room temperature. One hour later, sodium cyanoborohydride (0.2 g, 1.6 mmol, 2 eq.) was added portionwise (3x) over a period of 1.5 hrs (every 30 minutes). The reaction was stirred overnight at room temperature. Ethyl acetate and 1 N NaOH were added to the reaction with thorough mixing and the organic layer was separated, dried with MgSO<sub>4</sub>,  
25 filtered and evaporated to an oil. This material was purified using flash chromatography (flash silica, 80:20/ hexane:ether) to afford (1:1 mixture of cis and trans stereoisomers) **47**. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.6 (d, 2 H), 3.75 (s, 6 H), 3.7 (d, 4 H), 3.0 (m, 2 H), 2.65 (m, 3 H), 2.2 (m, 1 H), 1.7 (m, 2 H), 1.45 (m, 2 H), 1.25 (d, 3 H), 1.0 (m, 3 H), 0.8 (t, 3 H).

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Example 8

**Preparation of 5-Diphenylphosphinoyl-8-hydroxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene (58); and 5,8-bis(Diphenylphosphinoyl)-3-methyl-1,2,3,4-tetrahydronaphthalene (59)**

Compound **9** (0.5 g, 2.0 mmol) was dissolved in dioxane (100 mL) at 0°C. Triethylamine (6 mL) was added and the reaction mixture was stirred at 0°C for 30 mins, followed by the addition of diphenylphosphinyl chloride (0.84 mL, 4.4 mmol). The reaction mixture was stirred overnight at room temperature. NaHCO<sub>3</sub> and dichloromethane were then added with thorough mixing. The organic layer was separated, dried with MgSO<sub>4</sub>, filtered and the solvent was evaporated to a crude oil which was purified by flash chromatography (flash silica, 70:30/ hexane:ether) to yield **59** and **58**. Compound **59**: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.85 (d, 4 H), 7.8 (d, 4 H), 7.5 (m, 12 H), 6.8 (q, 2 H), 3.0 (m, 2 H), 2.4 (m, 7 H), 1.8 (m, 1 H), 1.4 (m, 4 H), 1.05 (d, 3 H), 0.85 (t, 6 H). Compound **58** (1:1 mix of regioisomers): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.85 (m, 4 H), 7.5 (m, 6 H), 6.6 (q, 1 H), 6.48 (s, 1 H), 6.21 (d, 1 H), 2.9 (m, 3 H), 2.3 (m, 9 H), 1.65 (m, 1 H), 1.4 (m, 1 H), 1.05 (d, 3 H), 0.85 (m, 6 H). In a similar manner, starting from **32**, was prepared both compounds **57** and **56**. In addition, in a similar manner, starting from **9**, was prepared compounds **49-51** using the appropriate reagents.

Example 9

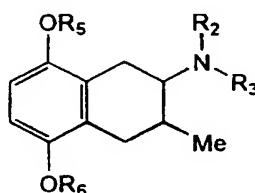
**Preparation of 5,8-Dimethoxy-*cis*-2-[*N*-(4-(2-(1-piperidinyloxy)ethoxy)phenyl)]amino-3-methyl-1,2,3,4-tetrahydronaphthalene (60)**

A solution of 1-(2-chloroethoxy)-4-nitrobenzene (2.01 g, 0.01 mol), piperidine (2.55 g, 0.03 mol), and toluene (10 mL) was refluxed overnight. The reaction mixture was filtered and the filtrate evaporated to give 2.97 g of 1-[2-(1-piperidinyloxy)ethoxy]-4-nitrobenzene as an orange oil, MS M<sup>+</sup> (m/e) 251.18. This material (1.25 g, 0.004 mol) was hydrogenated in ethanol (25 mL) in the

presence of 10% Pd-C catalyst at 40 psig and 25° C overnight. Filtration and evaporation of the reaction mixture gave 0.95 g of 4-[2-(1-piperidinyl)ethoxy]aniline as a light brown oil, MS M<sup>+</sup> (m/e) 221.24. A solution of this oil (0.220 g, 1.0 mmol), 3-methyl-5,8-dimethoxy-2-tetralone (**6**, 0.206, 0.94 mmol), and 1,2-dichloroethane (3.5 mL) was treated with sodium triacetoxymethylborohydride (0.300 g, 1.4 mmol) and acetic acid (0.060 g, 1.0 mmol) and the resulting mixture was stirred overnight at 25° C. The reaction was treated with 3N sodium hydroxide solution with vigorous stirring. The organic layer was separated, washed with saturated sodium chloride solution, dried over potassium carbonate, filtered and evaporated to a red oil. This material was purified by C-18 reverse phase high pressure liquid chromatography affording compound **60** as a pink solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.10-7.22 (m, 2H), 6.52-6.82 (m, 4H), 4.25-4.33 (m, 2H), 3.60-3.80 (m, 3H), 3.75 (s, 3H), 3.71 (s, 3H), 3.48-3.38 (m, 2H), 2.80-3.05 (m, 4H), 1.80-2.10 (m, 4H), 1.10-1.50 (m, 3H), 1.00 (d, 3H). MS M<sup>+</sup> (m/e) 425.38.

The compounds shown in Table 1, below, were prepared according to the procedures described herein.

**Table 1**



Cp.	R <sub>5</sub>	R <sub>6</sub>	R <sub>2</sub>	R <sub>3</sub>	Config.	MS M <sup>+</sup> (m/e)
9	H	H	Pr	Pr	trans	278.24
15	H	H	Pr	Pr	trans, ent.	278.25
16	H	H	Pr	Pr	trans, ent.	278.21
7	Me	Me	H	Pr	trans	
8	Me	Me	Pr	Pr	trans	306.28
17	Me	Me	Pr	Pr	trans, ent.	306.24
18	Me	Me	Pr	Pr	trans, ent.	306.27

Cp.	R <sub>5</sub>	R <sub>6</sub>	R <sub>2</sub>	R <sub>3</sub>	Config.	MS M <sup>+</sup> (m/e)
19	Me	Me	Pr	Ph(CH <sub>2</sub> ) <sub>3</sub>	trans	382.28
20	H	H	Pr	Ph(CH <sub>2</sub> ) <sub>3</sub>	trans	354.27
21	Me	Me	Pr	cC <sub>6</sub> H <sub>11</sub> CH <sub>2</sub>	trans	360.32
22	H	H	Pr	cC <sub>6</sub> H <sub>11</sub> CH <sub>2</sub>	trans	332.32
23	Me	Me		-(CH <sub>2</sub> ) <sub>2</sub> O(CH <sub>2</sub> ) <sub>2</sub> -	cis	292.66
24	H	H		-(CH <sub>2</sub> ) <sub>2</sub> O(CH <sub>2</sub> ) <sub>2</sub> -	cis	264.28
25	Me	Me	Pr	CH <sub>2</sub> Ph	trans	354.74
26	H	H	Pr	CH <sub>2</sub> Ph	trans	326.21
27	Me	Me	Pr	MeS(CH <sub>2</sub> ) <sub>3</sub>	trans	352.29
28	H	H	Pr	MeS(CH <sub>2</sub> ) <sub>3</sub>	trans	324.24
29	Me	Me	Pr	(2-thienyl)CH <sub>2</sub>	trans	360.24
30	H	H	Pr	(2-thienyl)CH <sub>2</sub>	trans	332.14
31	Me	Me	Pr	CH <sub>2</sub> CCH	trans	302.21
32	H	H	Pr	CH <sub>2</sub> CCH	trans	274.16
33	Me	Me	Pr	CH <sub>2</sub> CCH	cis	302.22
34	H	H	Pr	CH <sub>2</sub> CCH	cis	274.16
35	Me	Me	Pr	CH <sub>2</sub> (4-IPh)	trans	480.11
36	H	H	Pr	CH <sub>2</sub> (4-IPh)	trans	452.14
37	Me	Me	Pr	CH <sub>2</sub> (4-BuOPh)	trans	426.25
38	H	H	Pr	CH <sub>2</sub> (4-OHPh)	trans	342.16
39	Me	Me	Pr	CH <sub>2</sub> (2-ClPh)	trans	388.18
40	H	H	Pr	CH <sub>2</sub> (2-ClPh)	trans	360.12
41	Me	Me	Pr	CH <sub>2</sub> (4-MePh)	trans	368.29
42	H	H	Pr	CH <sub>2</sub> (4-MePh)	trans	340.18
43	H	X	Pr	Pr	trans	346.29
44	X	H	Pr	Pr	trans	346.30
45	Me	H	Pr	Pr	trans	292.22
46	H	Me	Pr	Pr	trans	292.22
47	Me	Me	Pr	CH(Me)CO <sub>2</sub> Me	cis & trans	350.26
48	Me	Me	H	CH(Me)CO <sub>2</sub> Me	cis & trans	308.19
49	Y	Y	Pr	Pr	trans	446.39
50	H	Y	Pr	Pr	trans	362.26
51	Y	H	Pr	Pr	trans	362.30
52	Me	Me	Pr	Me	trans	278.19
53	H	H	Pr	Me	trans	250.18
54	Me	Me	Pr	Z	trans	402.28
55	H	H	Pr	Z	trans	374.25
56	H	X'	Pr	CH <sub>2</sub> CCH	trans	474.13
57	X'	X'	Pr	CH <sub>2</sub> CCH	trans	674.11
58	H	X'	Pr	Pr	trans	478.21
59	X'	X'	Pr	Pr	trans	678.11
60	Me	Me	H	Y'	cis	425.38

X = -CH<sub>2</sub>CH=CMe<sub>2</sub>

Y = -C(O)-t-Bu

Z = -CH<sub>2</sub>CH<sub>2</sub>CH(Me)CH<sub>2</sub>CH<sub>2</sub>CH=CMe<sub>2</sub>X' = P(O)Ph<sub>2</sub>

Y' = 4-[(2-(1-piperidinyloxy)Ph

Example 10

10 As a specific embodiment of an oral composition, 100 mg of Compound 9 from Example 1 is formulated with sufficient finely divided lactose to provide a total amount of 580 to 590 mg to fill a size O hard gel capsule.

Example 11

5        **A $\beta$ <sub>1-42</sub> and A $\beta$ <sub>1-40</sub> binding to alpha-7 nAChRs can be demonstrated in competitive binding experiments with  $\alpha$ -bungarotoxin**

SK-N-MC cell membranes were incubated with 0.5 nM of <sup>125</sup>I- $\alpha$ -bungarotoxin in the presence of various concentrations of A $\beta$ <sub>1-42</sub> and A $\beta$ <sub>1-40</sub> at 25°C for 1 hr. The assay mixture was then rapidly filtered and the radioactivity  
10        that was retained on the filter was determined. These studies showed that both A $\beta$ <sub>1-42</sub> and A $\beta$ <sub>1-40</sub> inhibited <sup>125</sup>I- $\alpha$ -bungarotoxin binding in a concentration-dependent manner with IC<sub>50</sub> values of 1 pM and 100 pM, respectively.

Example 12

15        **A $\beta$ <sub>1-42</sub> binding to alpha-7 nAChRs is supported by competitive binding experiments with <sup>125</sup>I-A $\beta$ <sub>1-40</sub> and provides a seed for amyloid deposition and, thus, incipient plaque formation**

20        Alpha-7 nAChRs were immobilized on wheat germ agglutinin coupled yittrium SPA beads and allowed to incubate with <sup>125</sup>I-A $\beta$ <sub>1-40</sub> in the presence of various concentrations of cold A $\beta$ <sub>1-40</sub> or A $\beta$ <sub>1-42</sub>. The data showed that 0.1 femtoM of cold A $\beta$ <sub>1-42</sub> completely abolished the binding, suggesting that A $\beta$ <sub>1-42</sub> interacted with alpha-7 nAChRs with high affinity. However, in the presence of  
25        10 nM of cold A $\beta$ <sub>1-42</sub>, the total binding of <sup>125</sup>I-A $\beta$ <sub>1-40</sub> to alpha-7 nAChRs was dramatically increased. We attribute this increase of binding at higher concentrations to A $\beta$ <sub>1-42</sub>-induced precipitation of the A $\beta$  peptides on the membranes. Prolonged incubation at lower concentrations also revealed that total binding was increased in reactions even with 100 pM of cold A $\beta$ <sub>1-42</sub>. In  
30        control experiments, 80 pM <sup>125</sup>I-A $\beta$ <sub>1-40</sub> did not bind appreciably, along with 1 pM to 1 nM of A $\beta$ <sub>1-40</sub>, to 30  $\mu$ g of Bowes melanoma membranes. Thus, A $\beta$  deposition can occur on biological cell membranes containing alpha-7 nAChRs, but does not occur non-specifically on control membranes, suggesting that alpha-7 nAChRs can specifically seed the aggregation of  $\beta$ -  
35        amyloid in biological systems.

Example 13**A $\beta$ <sub>1-42</sub> binding to alpha-7 nAChRs is of higher affinity than A $\beta$ <sub>1-40</sub> binding to alpha-7 nAChRs**

Alpha-7 nAChRs were allowed to interact with A $\beta$ <sub>1-40</sub> or A $\beta$ <sub>1-42</sub> and the mixture was then immunoprecipitated with anti-alpha-7 nAChR antibodies. Western analyses of the immunoprecipitated proteins identified only the presence of A $\beta$ <sub>1-42</sub>, indicating that the A $\beta$ <sub>1-42</sub>/alpha-7 nAChR interaction is robust and of high affinity. Since it has already been shown that A $\beta$ <sub>1-40</sub> binds to the alpha-7 receptor, the failure in detecting A $\beta$ <sub>1-40</sub> in this co-precipitation experiment suggests that the A $\beta$ <sub>1-40</sub>/alpha-7 nAChR interaction is of lower affinity than the A $\beta$ <sub>1-42</sub>/alpha-7 nAChR interaction.

Example 14**Compound 9 inhibited A $\beta$  aggregation**

A $\beta$ s are known to form aggregates leading to the formation of amyloid plaques that are characteristic of Alzheimer's disease. This phenomenon can be demonstrated *in vitro* by using Synthaloid plates, coated with A $\beta$  crystallization centers, and labelled A $\beta$ s for detecting aggregation. We have validated this aggregation assay using both <sup>125</sup>I-A $\beta$ <sub>1-40</sub> and fluo-A $\beta$ <sub>1-40</sub> in a buffer containing 50 mM HEPES, pH 7.4, 0.1% BSA, 10% FCS and protease inhibitors. About 500 pM of <sup>125</sup>I-A $\beta$ <sub>1-40</sub> or 100 nM of fluo-A $\beta$ <sub>1-40</sub> in 100  $\mu$ l of each well of the 96-well Synthaloid plate was allowed to incubate for 2.5 hr at room temperature in the presence or absence of inhibitors. At the end of the incubation, unbound protein in the wells was removed by three washes using the above buffer. The amount of bound protein which represented A $\beta$  aggregation was measured either by scintillation counting or fluorescence measurements. Compound 9 was found to potently inhibit A $\beta$  aggregation with

10nM IC<sub>50</sub>. Time course studies also showed that prolonged incubation of amyloid aggregates with Compound 9 resulted in disaggregation.

Compounds which inhibit A $\beta$  aggregation with an IC<sub>50</sub> <100 micromolar may be effective in inhibiting the binding of A $\beta$  to alpha-7 in such a manner as to have a positive therapeutic effect useful for the treatment of neurodegenerative disorders.

#### Example 15

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#### **Compound 9 blocked the effect of A $\beta$ s on acetylcholine release from synaptosomes**

Synaptosomes from guinea pig hippocampus were incubated with 0.1  $\mu$ M <sup>3</sup>H-choline and then subjected to repeated washes to remove unincorporated <sup>3</sup>H-choline. The synaptosomes were treated with 65 mM K<sup>+</sup> for 30 seconds to elicit 3H-acetylcholine release. While A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> at 100 pM both inhibited acetylcholine release from these preparations (33% inhibition for both A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub>), pretreatment of the synaptosomes with both 10 nM of Compound 9 and 100 pM of either A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> prior to K<sup>+</sup> stimulation was found to have no effect on acetylcholine release.

#### Example 16

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#### **Compound 9 inhibited <sup>125</sup>I-A $\beta$ <sub>1-40</sub> binding to alpha-7 nAChRs**

To determine the effect of compound 9 on <sup>125</sup>I-A $\beta$ <sub>1-40</sub> to alpha-7 nAChRs, alpha-7 nAChR contained SK-N-MC cell membranes were immobilized on wheat germ agglutinin coupled yttrium SPA beads and allowed to incubate with <sup>125</sup>I-A $\beta$ <sub>1-40</sub> in the presence of various concentrations of compound 9. The result demonstrated that Compound 9 efficiently inhibited the binding of <sup>125</sup>I-A $\beta$ <sub>1-40</sub> to SK-N-MC cells with a 300 pM IC<sub>50</sub>. Compounds which inhibit the binding of A $\beta$  to alpha-7 nAChRs with an IC<sub>50</sub> <1 micromolar may have a

positive therapeutic effect useful for the treatment of neurodegenerative disorders.

### Example 17

5

#### **Assay for peptide binding to the 206-216 stretch of the human $\alpha 7$ nAChR**

A 2-5 ug quantity of the 11 amino acid peptide comprised of the 206-216 stretch of the  $\alpha 7$  nAChR (N-Ac, C(O)NH<sub>2</sub>) are added to a 96-well microtiter plate in 50 mL 10mM HEPES, pH 7.4, or any buffer 50ul. <sup>125</sup>I- $\beta$ -amyloid<sub>1-40</sub> (2000Ci/mmol, 50pM) was added to the wells in the presence and absence of inhibitors (1 uM to 10 uM) dissolved in 1uL of 30% or 100% DMSO. Unbound ligands were removed and bound radioactivity was measured using a Microbeta liquid scintillation counter. Ligand binding can be inhibited by  $\alpha$ -bungarotoxin, the peptide itself, and Compound 9.

15

Biological data for representative compounds of the present invention is provided in Table 2 which follows.

20

Table 2

Cp. #	% Inh Agg. (conc $\mu$ M)	% $\alpha 7$ nAChR 206-216 Binding	%ACh Release at 10 $\mu$ M (IC <sub>50</sub> , $\mu$ M)
9	>90 (10)	94	94 (0.1)
15	50-90 (10)	85	(0.25)
16	50-90 (10)	44	(10)
7	50(100)	NT	NT
8	50-90 (10)	NT	(0.7)
17	>90 (10)	NT	63 (1)
18	50-90 (10)	NT	70 (10)
19	30 (5)	NT	NT
20	39 (5)	NT	NT
21	20 (5)	NT	NT
22	38 (50)	NT	NT
23	NT	54	NT
24	NT	43	NT
25	NT	48	NT
26	<30 (10)	64	NT
27	NT	NT	NT
28	50-90 (10)	34	NT



Cp. #	% Inh Agg. (conc $\mu$ M)	% $\alpha$ 7 nAChR 206-216 Binding	%ACh Release at 10 $\mu$ M (IC <sub>50</sub> , $\mu$ M)
29	12 (10)	NT	NT
30	38 (5)	NT	NT
31	34 (10)	NT	NT
32	69 (10)	NT	NT
33	NT	NT	NT
34	69 (10)	NT	NT
35	38 (10)	NT	NT
36	<30 (10)	NT	NT
37	<30 (10)	NT	NT
38	<30 (10)	NT	NT
39	85 (10)	NT	NT
40	75 (10)	NT	NT
41	<30 (10)	19	14
42	60 (10)	NT	NT
43	50-90 (10)	10	0
44	50-90 (10)	23	0
45	30-50 (78)	NT	78
46	50-90 (10)	18	0
47	NT	NT	NT
48	<30 (10)	2	0
49	50-90 (10)	0	17
50	<30 (10)	0	67
51	30-50 (10)	0	54
52	60 (10)	0	0
53	50-90 (10)	0	18
54	50-90 (10)	0	0
55	50-90 (10)	0	15
56	<30 (10)	78	8
57	<30 (10)	63	11
58	<30 (10)	42	18
59	<30 (10)	72	25
60	very active	0	24

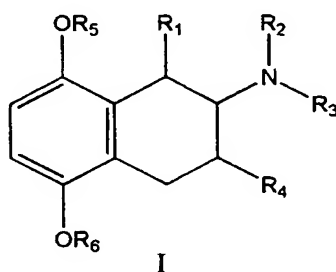
While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptations and/or modifications as come within the scope of the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. A method of treating a neurodegenerative disorder in a subject in need thereof which comprises administering to the subject an amount of a compound effective to inhibit the binding of an amyloid beta peptide with alpha-7 nicotinic acetylcholine receptors.
2. The method of Claim 1, wherein the alpha-7 nicotinic acetylcholine receptors are human alpha-7 nicotinic acetylcholine receptors.
3. The method of Claim 2, wherein the neurodegenerative disorder is selected from Alzheimer's disease, Pick's disease, diffuse Lewy body disease, progressive supranuclear palsy (Steel-Richardson syndrome), multisystem degeneration (Shy-Drager syndrome), motor neuron diseases including amyotrophic lateral sclerosis, degenerative ataxias, cortical basal degeneration, ALS-Parkinson's-Dementia complex of Guam, subacute sclerosing panencephalitis, Huntington's disease, Parkinson's disease, synucleinopathies, primary progressive aphasia, striatonigral degeneration, Machado-Joseph disease/spinocerebellar ataxia type 3 and olivopontocerebellar degenerations, Gilles De La Tourette's disease, bulbar and pseudobulbar palsy, spinal and spinobulbar muscular atrophy (Kennedy's disease), primary lateral sclerosis, familial spastic paraplegia, Werdnig-Hoffmann disease, Kugelberg-Welander disease, Tay-Sach's disease, Sandhoff disease, familial spastic disease, Wohlfart-Kugelberg-Welander disease, spastic paraparesis, progressive multifocal leukoencephalopathy, and prion diseases (including Creutzfeldt-Jakob, Gerstmann-Sträussler-Scheinker disease, Kuru and fatal familial insomnia, age-related dementia, vascular dementia, diffuse white matter disease (Binswanger's disease), dementia of endocrine or metabolic origin, dementia of head trauma and diffuse brain damage, dementia pugilistica or frontal lobe dementia, neurodegenerative disorders resulting from cerebral ischemia or infarction including embolic occlusion and thrombotic occlusion as well as intracranial hemorrhage of any type, intracranial and intravertebral lesions, hereditary cerebral angiopathy, nonneuropathic hereditary amyloid, Down's

syndrome, macroglobulinemia, secondary familial Mediterranean fever, Muckle-Wells syndrome, multiple myeloma, pancreatic- and cardiac-related amyloidosis, chronic hemodialysis arthropathy, or Finnish and Iowa amyloidosis.

4. The method of Claim 3, wherein the amyloid beta peptide is  $A\beta_{1-42}$ .
5. The method of Claim 3, wherein the neurodegenerative disorder is Alzheimer's disease.
6. The method of Claim 5, wherein the compound inhibits the binding of  $A\beta_{1-42}$  with the human alpha-7 nicotinic acetylcholine receptor by binding to  $A\beta_{1-42}$ .
7. The method of Claim 3, wherein the compound inhibits the binding of  $A\beta_{1-42}$  with the human alpha-7 nicotinic acetylcholine receptor by binding to human alpha-7 nicotinic acetylcholine receptors.
8. The method of Claim 3, wherein the compound inhibits the binding of  $A\beta_{1-42}$  with the human alpha-7 nicotinic acetylcholine receptor by inhibiting aggregation of amyloid beta peptides.
9. The method of Claim 5, wherein the compound is of the formula I



- wherein  $R_1$  is hydrogen or  $C_1-C_4$  alkyl;  
 $R_2$  is selected from hydrogen,  $C_1-C_6$  alkyl, aryl or  $C_7-C_{10}$  aralkyl;  
 $R_3$  is selected from hydrogen,  $C_1-C_6$  alkyl,  $C_3-C_{10}$  alkenyl,  
 $C_3-C_8$  cycloalkyl,  $C_1-C_6$  alkoxy carbonyl,  $C_1-C_6$  alkyl,

C<sub>1</sub>-C<sub>6</sub> alkylthio, heteroaryl, C<sub>1</sub>-C<sub>4</sub> alkyl, unsubstituted or substituted aryl or unsubstituted or substituted C<sub>7</sub>-C<sub>10</sub> aralkyl wherein the substituent on the aryl or aralkyl are one or more substituents independently selected from the group consisting of halogen, hydroxy, C<sub>1</sub>-C<sub>6</sub> alkyl and unsubstituted or substituted

- 5 C<sub>1</sub>-C<sub>6</sub> alkoxy wherein the substituents on the alkoxy are one or more substituents independently selected from amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>1</sub>-C<sub>6</sub> dialkylamino, pyrrolidinyl, piperidinyl, azepinyl or morpholinyl; or

- R<sub>2</sub> and R<sub>3</sub>, together with the nitrogen to which they are attached, form a five or six-membered heterocyclic ring selected from pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl or piperazinyl;
- 10

R<sub>4</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, or C<sub>7</sub>-C<sub>10</sub> aralkyl; and

R<sub>5</sub> and R<sub>6</sub> are each independently selected from hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>3</sub>-C<sub>10</sub> alkenyl, C<sub>1</sub>-C<sub>8</sub> alkylcarbonyl, or diphenylphosphinyl; and pharmaceutically acceptable salts and prodrugs thereof.

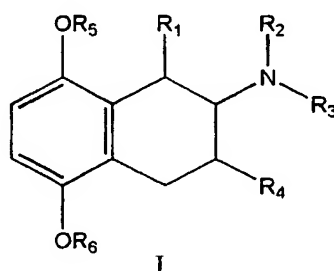
15

10. A method of treating and/or preventing dementia in an Alzheimer's disease patient which comprises administering to the subject a therapeutically effective amount of a compound which inhibits the binding of an amyloid beta peptide with alpha-7 nicotinic acetylcholine receptors.

20

11. The method of Claim 10, wherein the amyloid beta peptide is A $\beta$ <sub>1-42</sub> and the alpha-7 nicotinic acetylcholine receptors are human alpha-7 nicotinic acetylcholine receptors.

- 25 12. The method of Claim 11, wherein the compound is of the formula I



wherein R<sub>1</sub> is hydrogen or C<sub>1</sub>-C<sub>4</sub> alkyl;

$R_2$  is selected from hydrogen,  $C_1$ - $C_6$  alkyl, aryl or  $C_7$ - $C_{10}$  aralkyl;

$R_3$  is selected from hydrogen,  $C_1$ - $C_6$  alkyl,  $C_3$ - $C_{10}$  alkenyl,

$C_3$ - $C_8$  cycloalkyl,  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy carbonyl,  $C_1$ - $C_6$  alkyl,

$C_1$ - $C_6$  alkylthio, heteroaryl,  $C_1$ - $C_4$  alkyl, unsubstituted or substituted aryl or

- 5 unsubstituted or substituted  $C_7$ - $C_{10}$  aralkyl wherein the substituent on the aryl or aralkyl are one or more substituents independently selected from the group consisting of halogen, hydroxy,  $C_1$ - $C_6$  alkyl and unsubstituted or substituted  $C_1$ - $C_6$  alkoxy wherein the substituents on the alkoxy are one or more substituents independently selected from amino,  $C_1$ - $C_6$  alkylamino,  $C_1$ - $C_6$  dialkylamino, pyrrolidinyl, piperidinyl, azepinyl or morpholinyl; or
- 10

$R_2$  and  $R_3$ , together with the nitrogen to which they are attached, form a five or six-membered heterocyclic ring selected from pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl or piperazinyl;

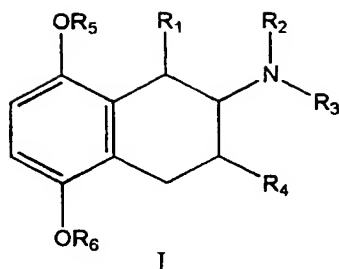
$R_4$  is  $C_1$ - $C_6$  alkyl, aryl, or  $C_7$ - $C_{10}$  aralkyl; and

- 15  $R_5$  and  $R_6$  are each independently selected from hydrogen,  $C_1$ - $C_6$  alkyl,  $C_3$ - $C_{10}$  alkenyl,  $C_1$ - $C_8$  alkyl carbonyl, or diphenylphosphinyl; and pharmaceutically acceptable salts and prodrugs thereof.

13. A method of improving memory and/or of halting the progression of mental  
20 deterioration in an Alzheimer's disease patient which comprises administering to the subject a therapeutically effective amount of a compound to inhibit the binding of an amyloid beta peptide with alpha-7 nicotinic acetylcholine receptors.

14. The method of Claim 13, wherein the amyloid beta peptide is  $A\beta_{1-42}$  and  
25 the alpha-7 nicotinic acetylcholine receptors are human alpha-7 nicotinic acetylcholine receptors.

15. The method of Claim 14, wherein the compound is of the formula I



wherein  $R_1$  is hydrogen or  $C_1$ - $C_4$  alkyl;

$R_2$  is selected from hydrogen,  $C_1$ - $C_6$  alkyl, aryl or  $C_7$ - $C_{10}$  aralkyl;

5         $R_3$  is selected from hydrogen,  $C_1$ - $C_6$  alkyl,  $C_3$ - $C_{10}$  alkenyl,  $C_3$ - $C_8$  cycloalkyl,  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy carbonyl,  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkylthio, heteroaryl,  $C_1$ - $C_4$  alkyl, unsubstituted or substituted aryl or unsubstituted or substituted  $C_7$ - $C_{10}$  aralkyl wherein the substituent on the aryl or aralkyl are one or more substituents independently selected from the group

10        consisting of halogen, hydroxy,  $C_1$ - $C_6$  alkyl and unsubstituted or substituted  $C_1$ - $C_6$  alkoxy wherein the substituents on the alkoxy are one or more substituents independently selected from amino,  $C_1$ - $C_6$  alkylamino,  $C_1$ - $C_6$  dialkylamino, pyrrolidinyl, piperidinyl, azepinyl or morpholinyl; or

15         $R_2$  and  $R_3$ , together with the nitrogen to which they are attached, form a five or six-membered heterocyclic ring selected from pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl or piperazinyl;

$R_4$  is  $C_1$ - $C_6$  alkyl, aryl, or  $C_7$ - $C_{10}$  aralkyl; and

20         $R_5$  and  $R_6$  are each independently selected from hydrogen,  $C_1$ - $C_6$  alkyl,  $C_3$ - $C_{10}$  alkenyl,  $C_1$ - $C_8$  alkyl carbonyl, or diphenylphosphinyl; and pharmaceutically acceptable salts and prodrugs thereof.

16.    A method for identifying compounds which are useful for the treatment of neurodegenerative disorders involving screening test compounds for their ability to block the interaction of a peptide selected from the group consisting

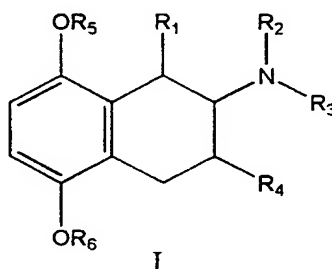
25    of  $^{125}\text{I}$ - $\text{A}\beta_{1-40}$ ,  $\text{A}\beta_{1-40}$  and  $\text{A}\beta_{1-42}$  with nicotine acetylcholine receptors.

17. The method of Claim 16, wherein the nicotine acetylcholine receptors are human alpha-7, human alpha-8, and/or human alpha-9 nicotinic acetylcholine receptors.

5 18. The method of Claim 17, wherein the nicotine acetylcholine receptors are human alpha-7 nicotine acetylcholine receptors.

19. The method of Claim 18, wherein the peptide is  $^{125}\text{I}$ -A $\beta_{1-40}$ .

10 20. A compound of the formula I:



wherein R<sub>1</sub> is hydrogen or C<sub>1</sub>-C<sub>4</sub> alkyl;

15 R<sub>2</sub> is selected from hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, aryl or C<sub>7</sub>-C<sub>10</sub> aralkyl;

R<sub>3</sub> is selected from hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>3</sub>-C<sub>10</sub> alkenyl,

C<sub>3</sub>-C<sub>8</sub> cycloalkyl, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy carbonyl, C<sub>1</sub>-C<sub>6</sub> alkyl,

C<sub>1</sub>-C<sub>6</sub> alkylthio, heteroaryl, C<sub>1</sub>-C<sub>4</sub> alkyl, unsubstituted or substituted aryl or

unsubstituted or substituted C<sub>7</sub>-C<sub>10</sub> aralkyl wherein the substituent on the aryl

20 or aralkyl are one or more substituents independently selected from the group consisting of halogen, hydroxy, C<sub>1</sub>-C<sub>6</sub> alkyl and unsubstituted or substituted

C<sub>1</sub>-C<sub>6</sub> alkoxy wherein the substituents on the alkoxy are one or more substituents independently selected from amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>1</sub>-C<sub>6</sub> dialkylamino, pyrrolidinyl, piperidinyl, azepinyl or morpholinyl; or

25 R<sub>2</sub> and R<sub>3</sub>, together with the nitrogen to which they are attached, form a five or six-membered heterocyclic ring selected from pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl or piperazinyl;

R<sub>4</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, or C<sub>7</sub>-C<sub>10</sub> aralkyl; and

$R_5$  and  $R_6$  are each independently selected from hydrogen,  $C_1$ - $C_6$  alkyl,  $C_3$ - $C_{10}$  alkenyl,  $C_1$ - $C_8$  alkylcarbonyl, or diphenylphosphinyl; and pharmaceutically acceptable salts and prodrugs thereof.

5 21. The compound of Claim 20, wherein

$R_1$  is hydrogen;

$R_2$  is selected from hydrogen or  $C_1$ - $C_4$  alkyl;

$R_3$  is selected from  $C_1$ - $C_4$  alkyl,  $C_3$ - $C_{10}$  alkenyl,

10  $C_5$ - $C_6$  cycloalkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_4$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_4$  alkyl,

$C_1$ - $C_6$  alkylthio, heteroaryl,  $C_1$ - $C_4$  alkyl, or unsubstituted or substituted

$C_7$ - $C_{10}$  aralkyl wherein the substituent on the aralkyl are one or two substituents independently selected from the group consisting of halogen, hydroxy,  $C_1$ - $C_4$  alkyl and unsubstituted or substituted  $C_1$ - $C_4$  alkoxy wherein the

15 substituents on the alkoxy are one or two substituents independently selected from amino,  $C_1$ - $C_4$  alkylamino,  $C_1$ - $C_4$  dialkylamino, pyrrolidinyl, or piperidinyl; or

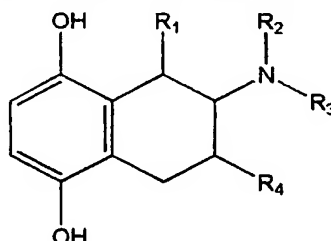
$R_2$  and  $R_3$ , together with the nitrogen to which they are attached, form a morpholinyl ring;

$R_4$  is  $C_1$ - $C_4$  alkyl; and

20  $R_5$  and  $R_6$  are each independently selected from hydrogen,

$C_1$ - $C_4$  alkyl,  $C_3$ - $C_6$  alkenyl,  $C_1$ - $C_6$  alkylcarbonyl, or diphenylphosphinyl; and pharmaceutically acceptable salts and prodrugs thereof.

22. The compound of Claim 20 of the formula



25

wherein  $R_1$  is hydrogen or  $C_1$ - $C_4$  alkyl;



R<sub>2</sub> and R<sub>3</sub> are each independently selected from hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, aryl or C<sub>7</sub>-C<sub>10</sub> aralkyl; and

R<sub>4</sub> is C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, or C<sub>7</sub>-C<sub>10</sub> aralkyl;  
and pharmaceutically acceptable salts and prodrugs thereof.

5

23. A compound of Claim 22 which is 5,8-dihydroxy-*trans*-2-di(*N*-propylamino)-3-methyl-1,2,3,4-tetrahydronaphthalene and pharmaceutically acceptable salts and prodrugs thereof.

10 24. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of Claim 20.

25. A pharmaceutical composition made by mixing a compound of Claim 20 and a pharmaceutically acceptable carrier.

15

26. A process for making a pharmaceutical composition comprising mixing a compound of Claim 20 and a pharmaceutically acceptable carrier.

20 27. A method for diagnosing Alzheimer's disease, monitoring the progression and prognosis of Alzheimer's disease and/or monitoring the therapeutic efficacy of any intervention or treatment of Alzheimer's disease comprising:

(a) obtaining a test sample from a subject wherein the test sample comprises circulating blood cells and/or olfactory neuroepithelial neuronal cell  
25 bodies or their neuronal processes; and

(b) analyzing the test sample for interaction of an amyloid beta peptide with alpha-7 nicotinic acetylcholine receptors.

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 31/135, 31/535, G01N 33/68,</b> <b>33/566, C07C 215/74</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 99/62505</b>  <b>(43) International Publication Date:</b> 9 December 1999 (09.12.99)
<b>(21) International Application Number:</b> PCT/US99/11702  <b>(22) International Filing Date:</b> 27 May 1999 (27.05.99)  <b>(30) Priority Data:</b> 60/087,577                      1 June 1998 (01.06.98)                      US  <b>(71) Applicant (for all designated States except US):</b> OR- THO-McNEIL PHARMACEUTICAL, INC. [US/US]; U.S. Route 202, Raritan, NJ 08869 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> REITZ, Allen, B. [US/US]; 109 Grenbrier Road, Lansdale, PA 19446 (US). DEMETER, David, A. [US/US]; 10657 Blackthorn Court, Fishers, IN 46038 (US). LEE, Daniel, H., S. [CA/US]; 38 Trailwood Drive, Northhampton, PA 18966 (US). WANG, Hoau-Yan [US/US]; 149 Kalos Street, Philadelphia, PA 19128 (US). CHEN, Robert, H. [US/US]; 39 Beverly Drive, Belle Mead, NJ 08502 (US). ROSS, Tina, Morgan [US/US]; 11 Culp Road, Audubon, PA 19403 (US). SCOTT, Malcolm, K. [US/US]; 1036 Beverly Way, Lansdale, PA 19446 (US). PLATA-SALAMAN, Carlos, R. [US/US]; 1313 Squire Drive, Ambler, PA 19002 (US).		<b>(74) Agents:</b> CIAMPORCERO, Audley, A., Jr. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08903-7003 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims</i> <i>and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 6 April 2000 (06.04.00)
<b>(54) Title:</b> METHOD FOR TREATING NEURODEGENERATIVE DISORDERS  <b>(57) Abstract</b>  The invention is directed to a method of treating a neurodegenerative disorder in a subject in need thereof which comprises administering to the subject an amount of a compound effective to inhibit the interaction of amyloid-beta with alpha-7 nicotinic acetylcholine receptors.		

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CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

Intern. and Application No  
PCT/US 99/11702

BNSDOCID: <WO 9982505A3 I >

# INTERNATIONAL SEARCH REPORT

Int. Appl. No.  
PCT/US 99/11702

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KIHARA ET AL.: "Neuroprotective effect of nicotine-related substances against beta-amyloid cytotoxicity" NEUROSCI. RES., vol. suppl., no. 21, 1997, pages s158 XP002128692 see abstract nr 1230	16-18,27
A	---	1-8,10, 11,13,14
Y	ITOH ET AL.: "Dysfunction of cholinergic and dopaminergic neuronal systems in beta-amyloid protein-infused rats" J. NEUROCHEM., vol. 66, 1996, pages 1113-1117, XP002916315 the whole document	16-18,27
A	---	1,3,5, 10,13
A	SALOMON ET AL.: "Nicotine inhibits amyloid formation by the beta-peptide" BIOCHEMISTRY, vol. 35, no. 42, 1996, pages 13568-13578, XP000857787 the whole document	1-8,10, 11,13, 14,16,27
A	MAURICE ET AL.: "Amnesia induced in mice by centrally administered beta-amyloid peptides involves cholinergic dysfunction" BRAIN RES., vol. 706, no. 2, 1996, pages 181-193, XP002128693 the whole document	1-8,10, 11,13, 14,16-18
A	AULD ET AL.: "Beta-Amyloid peptides as direct cholinergic neuromodulatorys : a missing link ?" TRENDS NEUROSCI., vol. 21, no. 1, January 1998 (1998-01), pages 43-49, XP000853850 cited in the application the whole document	1-8,10, 11,13, 14,16-18
A	ZAMANI ET AL.: "Nicotine modulates the neurotoxic effect of beta-amyloid protein(25-35) in hippocampal cultures" NEUROREPORT, vol. 8, no. 2, 20 January 1997 (1997-01-20), pages 513-517, XP002128694 the whole document	1-3,5, 10,13
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# INTERNATIONAL SEARCH REPORT

Int. l. Application No  
PCT/US 99/11702

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	<p>SJÖBERG ET AL.: "Neuronal nicotinic receptor activation : a promising strategy for the treatment of Alzheimer's disease ?"</p> <p>INT. J. GERIATRIC PSYCHOPHARMACOLOGY, vol. 1, no. 3, 1998, pages 145-149, XP002128695 the whole document</p>	1-3,5, 10,13
A	<p>CH 637 363 A (SANDOZ AG) 29 July 1983 (1983-07-29) the whole document</p>	20-26
A	<p>EP 0 360 077 A (TROPONWERKE GMBH &amp; CO KG) 28 March 1990 (1990-03-28) page 6, line 8 - line 36</p>	20-26
A	<p>MCDERMED ET AL.: "Synthesis and pharmacology of some 2-aminotetralins. Dopamine receptor agonists"</p> <p>J. MED. CHEM., vol. 18, no. 4, 1975, pages 362-367, XP002067369 see compounds 57,58,75 of table I pages 364-365</p>	20-26

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/11702

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim(s) 1-15,27  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:  
SEE FURTHER INFORMATION CPT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



## INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/1702

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

### Continuation of Box I.2

Present claims 1-2,4-8,10-11,13-14,16-19,27 relate to a method defined by the use of compounds characterized by reference to a desirable characteristic or property, namely the interference with the binding or interaction between alpha-7 nicotinic acetylcholine receptor and beta-amyloid peptide.

The claims cover all compounds/methods having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds/methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds of formula I.

Present claims 1- 3 relate to an extremely large number of possible diseases. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the diseases claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely Alzheimer's disease (see pages 18, 20 of description) and "neurodegenerative disorders" as a general term, regardless to any other specific disorder.

Due to the use of the term "prodrugs thereof", present claims 9, 11, 14, 20-26 relate to an extremely large number of possible compounds. In fact, the claims contain so many options, that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible (It is impossible to know which compounds/ structures are meant or included within the term "prodrugs"). Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely compounds of formula I as such and their pharmaceutically acceptable salts.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/11702

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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